UNIVERSIDAD AUTÓNOMA DE MADRID
Facultad de Ciencias
Departamento de Química Analítica y Análisis Instrumental

ASSESSMENT OF ARSENIC OCCURRENCE IN DIFFERENT MINING ENVIRONMENTS BY THE DEVELOPMENT AND APPLICATION OF SUITABLE ANALYTICAL METHODOLOGIES

European Ph.D. Dissertation

RAQUEL LARIOS ARDILA

Madrid, 2012

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DEPARTAMENTO DE TECNOLOGÍA
División de Química
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MARÍA ISABEL RUCANDIO SÁEZ, Doctora en Ciencias Químicas, Investigador Titular y Jefa de la Unidad de Espectroscopía y **RODOLFO FERNÁNDEZ MARTÍNEZ**, Doctor en Ciencias Químicas e Investigador, ambos pertenecientes al Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT).

HACEN CONSTAR:

Que la licenciada Dª **RAQUEL LARIOS ARDILA**, ha realizado bajo su dirección el presente trabajo de investigación titulado: **"Assessment of arsenic occurrence in different mining environments by the development and application of suitable analytical methodologies"** fundamentalmente en los laboratorios de la Unidad de Espectroscopía de la División de Química del Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), constituyendo la Memoria de Tesis Doctoral de su autora.

Así mismo, manifiestan que el trabajo descrito en la presente Memoria reúne todos los requisitos para su defensa y aprobación, por lo que autorizamos su presentación para que sea defendido como **Tesis Doctoral con Mención de Doctorado Europeo** en el Departamento de Química Analítica y Análisis Instrumental de la Facultad de Ciencias de la Universidad Autónoma de Madrid**.**

Y para que así conste, y a los efectos oportunos, firmamos el presente escrito en Madrid, a 11 de Noviembre de 2011

Fdo. Mª Isabel Rucandio Sáez Fdo. Rodolfo Fernández Martínez

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Doña MARIA DOLORES PETIT DOMÍNGUEZ, profesora titular del Dpto. de Química Analítica y Análisis Instrumental de la Universidad Autónoma de Madrid, como ponente y tutora de la tesis doctoral de Dª. **RAQUEL LARIOS ARDILA,**

INFORMA

Que el trabajo realizado para aspirar al grado de Doctor titulado **"Assessment of arsenic occurrence in different mining environments by the development and application of suitable analytical methodologies"** reúne las condiciones necesarias para ser presentado como memoria original.

En él se lleva a cabo un estudio del **impacto medioambiental** producido por el arsénico procedente de instalaciones mineras abandonadas en Asturias. Se evalúa la movilidad y disponibilidad del arsénico en suelos, sedimentos, aguas superficiales y algunas plantas autóctonas de esta zona minera, de manera que se pueda conocer la potencial toxicidad de este elemento para los organismos vivos.

Para ello se ha **desarrollado y validado un nuevo método de extracción secuencial** que se caracteriza por su simplicidad. Este método permite cuantificar el arsénico presente en suelos y sedimentos y que se encuentra asociado a fracciones operacionalmente definidas. Se evalúa la selectividad y viabilidad de cada agente extractante, así como la interacción entre las diferentes etapas; optimizando las condiciones experimentales en que debe llevarse a cabo cada etapa de extracción, para mejorar la operatividad del método y facilitar así su aplicación en el laboratorio. De esta manera se puede obtener la máxima información sobre la movilidad, distribución y geoquímica del arsénico en muestras medioambientales (suelos y sedimentos); evaluando la influencia de los principales factores ambientales: pH, potencial redox, contenido en materia orgánica, etc. La viabilidad del método propuesto se demostró mediante su aplicación a una amplia variedad de muestras reales.

El método propuesto **se compara con otros protocolos de extracción secuencial tradicionales**, destacando la mejora que proporciona el nuevo método al ofrecer un patrón de distribución de arsénico en suelos y sedimentos más adecuado.

Por otro lado, se han **desarrollado y validado nuevas metodologías analíticas** que permiten la **determinación de diferentes especies de arsénico en aguas y plantas** mediante el uso de técnicas acopladas como HPLC-HG-AFS y HPLC-ICP-MS respectivamente. Los resultados permiten conocer en estas muestras medioambientales la distribución y movilidad de diferentes especies de arsénico como As (III), As (V), el ácido monometilarsénico y el ácido dimetilarsénico.

Todo el trabajo presentado se ha realizado con rigor, siguiendo la metodología científica apropiada y los resultados obtenidos cubren perfectamente los objetivos planteados, por lo que el trabajo puede considerarse concluido y pueden iniciarse los trámites necesarios para la defensa pública y consecución del Título de Doctor con mención “Doctorado Europeo”.

Y para que conste a los efectos oportunos, firma el presente informe en Madrid a 21 de Noviembre de 2011.

Fdo: Mª Dolores Petit Domínguez

A mis padres

“Le problème avec notre époque est
que le futur n'est plus ce qu'il était!”

(“¡El problema de nuestros tiempos es
que el futuro ya no es lo que era!”)

PAUL VALÉRY

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Agradezco al Ciemat la beca que recibí para realizar la tesis doctoral, y al proyecto Geocima la oportunidad de formar parte del mismo.

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ABSTRACT

Arsenic is widespread in some regions of Asturias (Northern Spain) as a consequence of the abandonment of the facilities of some cinnabar mines after their closures and the currently open-air state of their corresponding spoil heaps. Natural weathering or variations in physicochemical parameters at the disposal site can alter the stability of waste materials, mobilizing mercury, arsenic and related heavy metals to the surrounding soils and sediments, provoking their solubilisation into water and the possible assimilation by the autochthon plants.

The current Doctoral Thesis pretends to perform a study of the environmental impact provoked by arsenic in some cinnabar mining areas through the assessment of its potential mobility and availability in soils and sediments, in their surrounding surface waters and in some autochthon plants growing in those sites.

Extremely high total arsenic concentrations were found in the studied soils, sediments and waste samples from the considered mine sites. Arsenic mobility is largely dependent on its associations with solid phases. With the aim of adequately evaluate arsenic mobility in soils and sediments, a novel sequential extraction procedure (SEP) has been developed and optimized for the study of arsenic fractionation in environmental solid samples. It pretends to sequentially target the most important solid phases scavenging arsenic, with special emphasis on the most labile fractions. The selectivity of the selected extracting agents was assured through experiments with natural and synthetic matrices of As-bearing minerals and arsenic-spiked materials. The protocol was proved to be applicable to variety of samples with different arsenic pollution sources. The proposed SEP was advantageously compared with other protocols currently applied to study arsenic and heavy metals mobility, since it offers a more appropriate arsenic distribution pattern. The application of this protocol to sediments and waste samples from the studied mining areas evidenced that the mobility of arsenic was dependent on the mineralogical and physicochemical characteristics of the site and on the extent of mining operations.

The surrounding surface waters of the studied mine sites exhibited high arsenic concentrations. In water, arsenic speciation determines arsenic mobility, since As(III) is more mobile than As(V). The study of arsenic speciation in surrounding waters of those mines was performed by using the hyphenated technique HPLC-HG-AFS. As(V) was practically the only species found in all the tested waters. Different preservation

procedures for arsenic speciation were tested, and no differences were found among the samples stored without any additive and those preserved with HCl or EDTA. An assessment of the factors controlling arsenic solubilisation in each mine site was performed based on physicochemical characterization and statistical studies of waters. Results showed that arsenic mobilization was largely in accordance with the mineralogy of each area. An investigation about possible seasonal variations of arsenic and other parameters influencing its mobility showed that they were not significantly dependent on the rainfall regime.

Plants growing in the studied mine sites presented high arsenic accumulation in their tissues. For those autochthon plants the study of arsenic speciation was performed by HPLC-ICP-MS. For the extraction of arsenic species (As (III), As(V), MMA, DMA) from the plant tissues a novel extraction methodology was developed and optimized. It achieved excellent extraction efficiencies, even for plants containing extremely high arsenic contents, with proved no interconversion of species. As(V) was the predominant species, and roots were found responsible for accumulating the most arsenic. The extent of arsenic accumulation by the plants was proved to be dependent on both the amount of bioavailable arsenic in their corresponding soils, and the plant species in question.

RESUMEN

El arsénico se encuentra ampliamente distribuido en algunas regiones de Asturias (Norte de España) como consecuencia del abandono de las instalaciones de algunas minas de cinabrio tras su cierre y el actual estado al aire libre de sus correspondientes escombreras. La erosión natural y/o variaciones en las condiciones ambientales del emplazamiento pueden alterar la estabilidad de los residuos, movilizando mercurio, arsénico y otros metales pesados relacionados desde los suelos y sedimentos circundantes, provocando su disolución en aguas y su posible asimilación por parte de plantas autóctonas.

El presente trabajo de Tesis Doctoral pretende llevar a cabo un estudio del impacto ambiental provocado por arsénico en algunas zonas mineras de Asturias a través de la evaluación de su movilidad y disponibilidad potencial en suelos y sedimentos de estos emplazamientos mineros, en las aguas superficiales circundantes y en algunas plantas autóctonas que crecen en esas áreas.

En los suelos, sedimentos y residuos de las minas estudiadas se hallaron concentraciones extremadamente elevadas de arsénico total. La movilidad del arsénico depende en gran medida de sus asociaciones con las fases sólidas de suelos y sedimentos. Por ello, con el propósito de evaluar adecuadamente la movilidad del arsénico se ha desarrollado y optimizado un nuevo método de extracción secuencial (SEP) para el estudio de su fraccionamiento en muestras sólidas medioambientales. Con él se pretende la extracción sucesiva de las fases sólidas más importantes responsables de la retención de arsénico en suelos y sedimentos, con especial énfasis en las fracciones más lábiles. La selectividad de los agentes extractantes seleccionados para la extracción de cada fase fue comprobada mediante experimentos con matrices, tanto naturales como sintéticas, de minerales de arsénico y de matrices dopadas con arsénico. Se demostró que el método resultaba viable para su aplicación a muestras cuyas fuentes de contaminación de arsénico eran diversas. El método propuesto resultó ventajoso de su comparación con otros dos protocolos comúnmente utilizados para el estudio de la movilidad de arsénico y metales pesados, ya que éste ofrece un patrón de distribución de arsénico más adecuado. La aplicación de este protocolo a sedimentos y muestras de residuos de las zonas mineras de estudio evidenciaron que la movilidad de arsénico depende de las características mineralógicas y fisicoquímicas del lugar, y del alcance de las operaciones mineras llevadas a cabo.

Las aguas superficiales de las proximidades de los yacimientos estudiados mostraron elevadas concentraciones de arsénico. La movilidad del arsénico en las aguas está determinada por su especiación, ya que el As(III) es más móvil que el As(V). El estudio de la especiación de arsénico en las aguas circundantes a las minas se llevó a cabo mediante las técnicas acopladas HPLC-HG-AFS. Prácticamente todo el arsénico encontrado en las aguas analizadas se encontraba en forma de As(V). Por otro lado, se probaron distintos procedimientos de conservación de la especiación de arsénico y no se hallaron diferencias significativas entre las muestras almacenadas sin ningún aditivo o aquellas adicionadas con HCl o EDTA. Se realizó una evaluación de los factores que controlan el proceso de disolución de arsénico en las aguas de cada área minera en base a una caracterización fisicoquímica y a estudios estadísticos de estas aguas. Los resultados demostraron que la movilización de arsénico era en gran medida concordante con la mineralogía de cada zona. Una investigación sobre las posibles variaciones estacionales de arsénico y otros parámetros que influyen en su movilidad demostró que no eran significativamente dependientes del régimen de precipitaciones.

Las plantas que crecen en las áreas mineras de estudio presentaron una elevada acumulación de arsénico en sus tejidos. El estudio de la especiación de arsénico en las plantas autóctonas fue realizado mediante HPLC-ICP-MS. Para la extracción de las especies de arsénico (As(III), As(V), MMA, DMA) de los tejidos vegetales se desarrolló y optimizó una nueva metodología. Con ella se logró una eficiencia en la extracción excelente, incluso cuando se aplicó a plantas con contenidos de arsénico extremadamente elevados, y se demostró que no se produce interconversión de las especies. Respecto a la especiación en las plantas estudiadas, el As(V) resultó ser la especie predominante, y las raíces fueron las responsables de la mayor parte de la acumulación de arsénico. Se demostró que el grado de acumulación de arsénico por las plantas depende tanto de la cantidad de arsénico biodisponible en los suelos correspondientes como de las especies vegetales en cuestión.

CONTENTS

1. INTRODUCTION

1.1 HISTORICAL BACKGROUND

The word *arsenic* was borrowed from the Syriac word ܐܠ ܙܪܢܝܩܐ *(al) zarniqa* [1] and the Persian word زرنیـــخ *Zarnikh*, meaning “yellow orpiment”, into Greek as *arsenikon* (Αρσενικόν). It is also related to the similar Greek word *arsenikos* (Αρσενικός), meaning "masculine" or "potent". The word was adopted in Latin *arsenicum* and Old French *arsenic,* from which the English word *arsenic* is derived [1].

During the Bronze Age, arsenic was often included in bronze, which made the alloy harder, resulting in the so-called "arsenical bronze" [2]. Arsenic sulphides (orpiment, realgar) and oxides have been known and used since ancient times [3]. In the 4th century BC, the Greek philosopher Aristotle referred to a substance which was probably the arsenic sulphide mineral realgar (AsS), and Zosimos (circa 300 AD) describes roasting *sandarach* (realgar) to obtain *cloud of arsenic* (arsenious oxide), which then is reduced to metallic arsenic [4].

It is believed that Albertus Magnus (Fig. 1.1) was the first to isolate this element in 1250 by heating soap together with arsenic trisulphide [5]. However, the medieval physician Paracelsus was the first who provided the detailed recipes for the preparation of elemental arsenic [6]. In 1649, Johann Schröder published two ways of preparing arsenic. Cadet’s fuming liquid (impure cacodyl), often claimed as the first synthesized organometallic compound, was synthesized in 1760 by Louis Claude Cadet de Gassicourt by the reaction of potassium acetate with arsenic trioxide [7].

The alchemical symbol of arsenic was the following:  .

During the Victorian era, arsenic (the “white arsenic” form, the arsenolite mineral, As_2O_3) was mixed with vinegar and chalk and eaten by women to improve the complexion of their faces, making their skin paler to show they did not work in the fields. Arsenic was also rubbed into the faces and arms of women to "improve their complexion" (Fig. 1.2).

As the symptoms of arsenic poisoning were roughly ill-defined, it was frequently used for murder from the imperial Rome until the advent of the Marsh test, a sensitive chemical test for its presence (another less sensitive but more general test is the Reinsch test). The arsenic species used by assassins and murderers generally

refers to As_2O_3 [6]. Owing to its use by the ruling class to murder and its potency and discreetness, arsenic has been called the *Poison of Kings* and the *King of Poisons* [8].

Fig. 1.1. Picture of Albertus Magnus (1193–1280), also known as Albert the Great, As discoverer.

Fig. 1.2. Portrait of a Victorian Woman. *Lady Lilith,* (a Victorian adaptation of the pre-Biblical Lilith) by Dante Gabriel Rossetti.

Perhaps the most famous person believed to have been a victim of arsenic poisoning was the French emperor Napoleon Bonaparte. After his final defeat at the Battle of Waterloo, Napoleon was imprisoned on the remote Island of St Helena. His death several years later, from what was officially recorded as stomach cancer, has proved controversial. Some writers have claimed that his death was the result of arsenic poisoning and cite evidence from analysis of what is believed to be a lock of his hair showing elevated levels of arsenic. If true, this could have been the result of deliberate poisoning, but a more intriguing argument centres on the wall decorations of Napoleon's quarters on the island. These reportedly included green-coloured wallpapers, which were very fashionable at the time. The pigments used to impart the green colour in such papers and paints were made from arsenic compounds. Stories of sickness amongst servants and others living alongside Napoleon lend credence to this hypothesis. The problem of arsenic ingestion, given that even the most desperate of prisoners is unlikely to eat his prison walls, has been ingeniously solved by suggesting that the wallpaper in that humid Atlantic Ocean climate was attacked by a mold, causing tiny fragments to break off and be inhaled. Whatever killed Napoleon, it seems likely that some of his contemporaries were poisoned by their fashionable salon decorations [6].

The use of arsenic as poison has also been very recurrent in novels and films. For instance, when in his play *Under Milk Wood (Bajo el bosque lácteo* in Spanish*)*, the

Welsh poet and humorist Dylan Thomas created the henpecked husband character of Mr Pugh, a man whose favourite reading was drawn from *Lives of the Great Poisoners*, it was clear that the poison of his fantasies was arsenic (…whispering as he took breakfast tea to his wife's room "Here's your arsenic, dear. And your weedkiller biscuit… Here's your… nice tea, dear") [6] (Fig. 1.3). In the film *Arsenic and Old Lace* (translated into Spanish as *Arsénico por compasión*), a crazy black comedy directed by Frank Capra and starring Cary Grant and Priscilla Lane in 1944, two "kind-hearted old ladies" relieve the presumed suffering of lonely old bachelors serving them elderberry wine spiked with arsenic, strychnine and "just a pinch of cyanide" (Fig. 1.4).

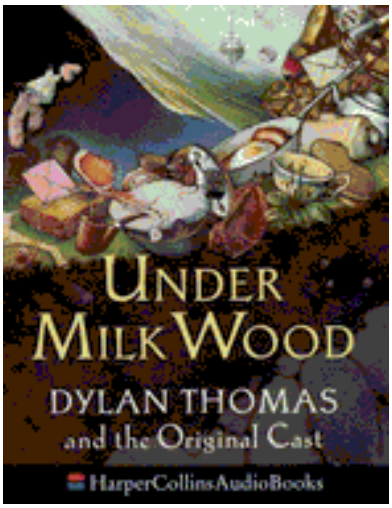

Fig. 1.3. Cover of the book *Under the Milk*, by Dylan Thomas.

Fig. 1.4. Poster of the film *Arsenic and Old Lace*, by Frank Capra.

1.2 ARSENIC CHARACTERISTICS

1.2.1 Chemical and physical properties

Arsenic is a chemical element with the symbol As, whose atomic number is 33 and its relative atomic mass is 74.92. It belongs to V-A group in the periodic table and it is seated beneath nitrogen and phosphorus. Due to its position in this group, arsenic can be considered as a metalloid since it exhibits properties of both metals and non-metals. The electron configuration for neutral arsenic is [Ar] $3d^{10}4s^{2}4p_{x}^{1}4p_{y}^{1}4p_{z}^{1}$, a state that supplies up to five valence electrons for participation in chemical bonding and empty *p* orbitals for electron occupation. Its most important chemical and physical properties are shown in Fig. 1.5.

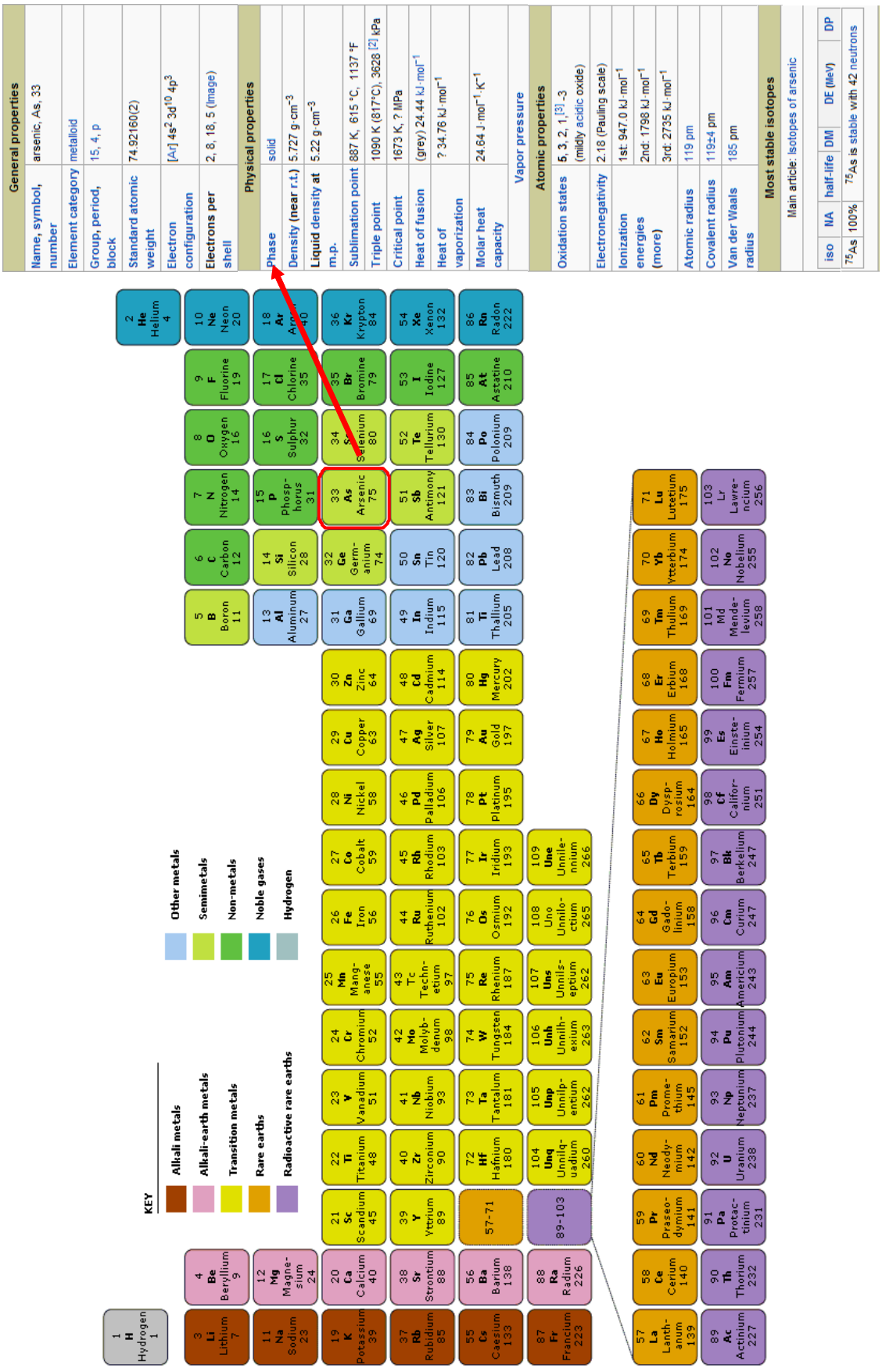

Fig. 1.5. Periodic table and arsenic properties.

Arsenic can exist in various allotropic forms, including grey or gamma-arsenic, yellow or alpha-arsenic and black or beta-arsenic, with grey being the most common allotrope. Grey arsenic adopts a double-layered structure consisting of many interlocked ruffled six-membered rings. Nearest and next-nearest neighbours form a distorted octahedral complex, with the three atoms in the same double-layer being slightly closer than the three atoms in the next layer [9]. This relatively close packing leads to a high density of 5.73 g/cm^3 [10]. It is brittle solid semiconductor and with a Mohs hardness of 3.5. Yellow arsenic is soft and waxy, and roughly similar to P_4. Both have four atoms arranged in a tetrahedral structure in which each atom is bound to the other three atoms by a single bond. This unstable and molecular allotrope is the most volatile, the least dense and the most toxic. Solid yellow arsenic is produced by rapid cooling of arsenic vapour, As_4. It is rapidly transformed into the grey arsenic by light. The yellow form has a density of 1.97 g/cm^3 [10]. Black arsenic is similar in structure to red phosphorus [10].

Naturally occurring arsenic is composed of one stable isotope, ^{75}As, although at least other 33 radioisotopes have also been synthesized, ranging its atomic mass from 60 to 92 [11].

The electron configuration for neutral arsenic stabilizes formal oxidation states from +5 to –3. Hence, its most common oxidation states are -3, 0, +3 y +5.

The most important compound containing arsenic in the oxidation state -3 is the arsine gas, AsH_3, a strong reducing agent. Some other arsines and methylarsines also correspond to the oxidation state -3, and they are unstable in oxidant environments (Fig. 1.6). The arsenides Na_3As, Ca_3As_2, Zn_3As_2, AlAs, AsNi or the sulphoarsenide SAsFe are other compounds containing arsenic in this oxidation state found in nature.

Elemental arsenic (oxidation state 0) is formed through arsenic oxides reduction. Nevertheless, given the affinity of arsenic to bond with other elements and species, native arsenic is rarely found in nature with the occasional exception in hydrothermal ores.

The structural configuration of As^{3+} has three pyramidal bonds and a lone electron pair occupying the fourth arm of a tetrahedron. Spectroscopic evidence shows that arsenite retains this pyramidal structure in solution with three oxygen ligands and the lone electron pair [12]. As well as the arsenite oxyanion, other arsenic compounds

with this oxidation state are usually found in aqueous solution, as As_2O_3, the AsO^+ cation or the corresponding hydroxide.

As^{5+} forms a regular tetrahedron when bonded to oxygen ligands, forming the stable arsenate $[AsO_4]^{3-}$ anion, similar in structure to the orthophosphate anion $[PO_4]^{3-}$. Its chemistry in solution also has similarities to phosphoric acid (H_3PO_4), with the important exception of the greater oxidizing potential of arsenate compared to phosphate [13]. As well as the arsenate oxyanion, the chemical form As_2O_5 is also very usual for this oxidation state.

The ability of arsenic to shift from an electropositive state, such as in oxyanions, entails that arsenic is most frequently found in the environment in the oxidation states +3 and +5 as arsenite (As(III)) and arsenate (As(V)) [14].

Although arsenic can bind covalently to most metals and non-metals, it predominantly bonds to oxygen and sulphur in nature, and is also able to form part of stable organic molecules [15]. The occurrence of natural compounds of arsenic is further discussed in the following section.

1.2.2 Arsenic species and compounds

Arsenic has diverse chemical behaviour in the natural environment. Its ability to readily change the oxidation state and bonding configuration confers to arsenic rich inorganic and organic chemistry. This behaviour is a consequence of the electron configuration of its valence orbitals, with partially filled states capable of both electron donation and overlap in covalent bonds. In natural compounds, arsenic bonds primarily to oxygen and sulphur, generating a variety of aqueous species and minerals. The affinity of arsenic for these two elements, along with its stable bonding to methyl groups, constitutes the structural basis for most organic compounds [16]. Fig. 1.6 exhibits the names and chemical structures of the predominant natural arsenic compounds in the environment.

Arsine gas (AsH_3), which is formally the most reduced form of arsenic (its oxidation state is –3), is notable for its extreme toxicity. It is a volatile gas that is only slightly soluble in water. Because of its volatility, its importance in the environment as a microbiological end product may be overlooked [14, 17].

Inorganic arsenic species are the predominant forms in aqueous solutions, especially the oxyanions arsenite and arsenate. Hence, inorganic arsenic species are

the main forms present in water used for human consumption and, to a lesser extent, in foodstuff [18].

Similar to nitrogen and phosphorus, arsenic has rich organic chemistry, due to its high ability to bind to a variety of organic ligands with different coordination geometries. A large number of organoarsenic compounds are found in the environment as a consequence of a variety of biomethylation processes and other biosynthetic pathways [14].

One of the most common classes of natural organoarsenic compounds are methylated forms of As(III) and As(V), such as the mono- and dimethyl oxoacids in their simplest forms, which can be generated by replacing a hydroxyl (-OH) ligand by a methyl group ($-CH_3$) in inorganic arsenate or arsenite structures [16]. They are commonly known as MMA (although some authors prefer to call it MA [19] and in this way is expressed in Fig. 1.6) and DMA respectively (Fig. 1.6).

Methylation of inorganic arsenic by microorganisms has been known since the 1930s, and most natural arsenolipids and arsenosugars detected in the environment are thought to be end products of arsenic detoxification by microorganisms and invertebrates [17, 20]. A general detoxification pathway for inorganic arsenate (aqueous $H_2AsO_4^-$ or $HAsO_4^{2-}$) by microorganisms, all enzymatically assisted, is thought to be: an initial reduction to arsenite; methylation and reoxidation to mono- and dimethyl oxoacids (methylarsonate (MMA) and dimethylarsinate (DMA), the metabolites more commonly found in the environment); possibly final reduction and methylation to trimethylarsine oxide, dimethylarsine, or trimethylarsine, volatile forms of arsenic that are difficult to detect [16, 17, 20].

One or more of the methyl or hydroxyl groups around arsenic can be replaced by other organic ligands or larger organic moieties such as sugars, lipids, or cyclic groups to create a wide variety of arsenic-bearing organic compounds (Fig. 6). Among them, arsenobetaine, arsenocholine and some other arsenosugar compounds are of environmental interest since their occurrence in fish and other aquatic fauna and flora has been shown in several studies [21-28]. It is noteworthy that arsenobetaine, which is the most commonly reported organoarsenical in marine animals, is virtually absent in the analyzed vertebrate and invertebrate freshwater organisms [26]. This represents the main difference in arsenic speciation between marine and freshwater organisms. Recently, arsenolipids have been found in cod fish oil; causing human health concerns [29, 30].

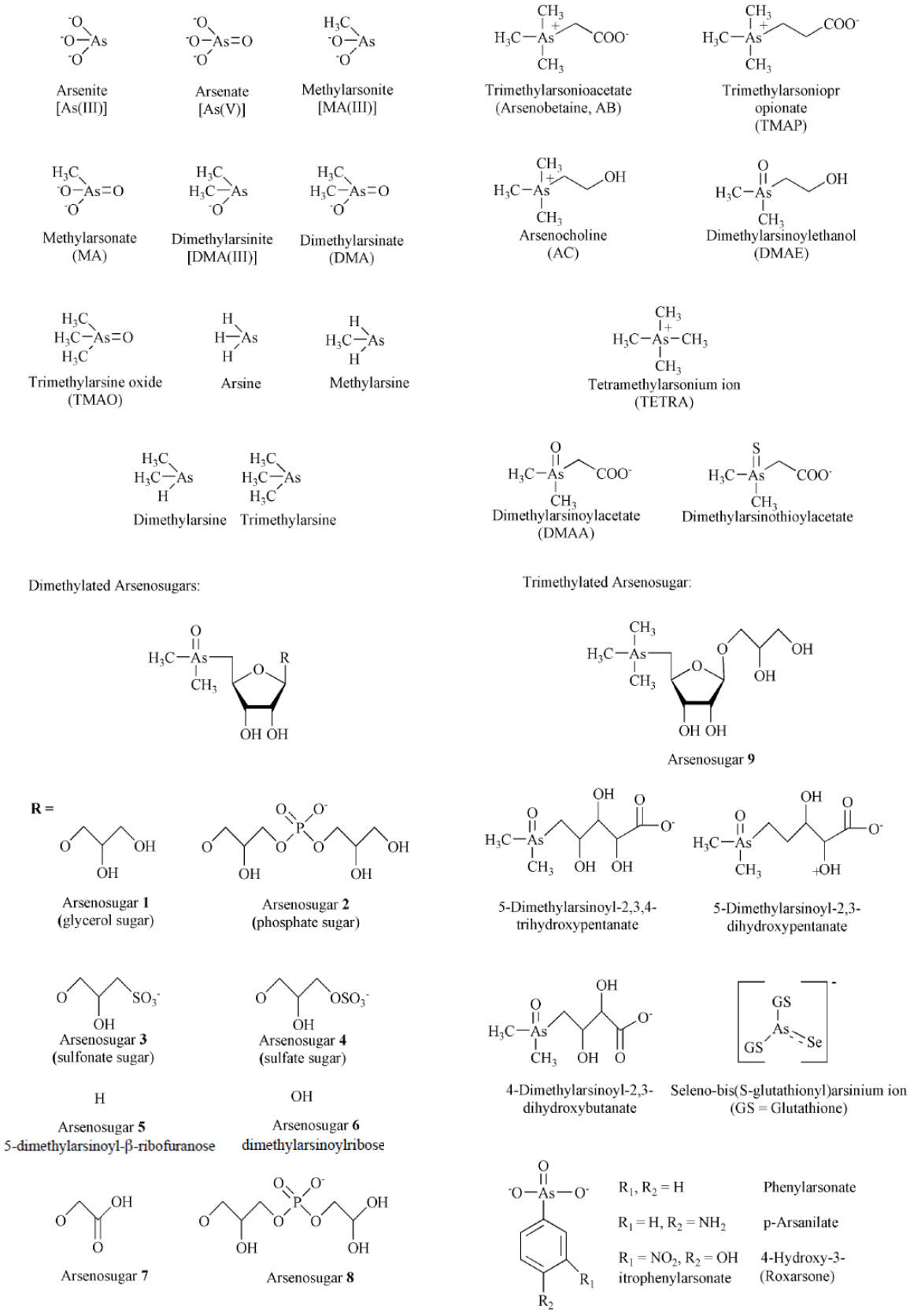

Fig. 1.6. Chemical structures with names, abbreviations and/or structure numbers for arsenic species of relevance [31].

1.2.3 Toxicological and biological aspects

The toxicity of arsenic strongly depends upon the chemical speciation. The effects of pH, Eh, adsorbing surfaces, biological mediation, organic matter, and key inorganic substances such as sulphide and phosphate combine in a complex and interwoven dynamic fashion to produce unique assemblages of arsenic species. The number of different arsenic species found in environmental samples and an understanding of the transformations between arsenic species have increased over the past few decades as a result of the development and application of new and refined analytical methods [30].

A typical statement in the literature is that inorganic arsenic species are more harmful and toxic than the organic compounds. Among them, As (III) is more toxic than As(V), due to its higher mobility [32-34]. Regarding organic compounds, methylated arsenic species have been reported to be much less toxic than inorganic compounds, and trivalent organic arsenicals are more toxic to mammals and aquatic species than the pentavalent forms [35]. Arsenosugars seem not be toxic [6]. These statements can be deduced from results of a standard test of toxicity, the commonly known 'LD 50' test, which establishes the dose necessary to kill 50% of the essayed individuals. Fig. 1.7 shows the amount, in milligrams per kilogram bodyweight, of particular arsenic compounds required to kill 50% of a test population of rats. The most toxic form of arsenic is the gas arsine (AsH_3), followed by the arsenite (AsO_3^{3-}) and then the arsenate (AsO_4^{3-}) forms. The organic (methylated) arsenic compounds [e.g. $(CH_3)_3As$] are much less toxic, and certain arsenic compounds such as the arsenosugars are not toxic. It is important, however, to emphasize that there is still considerable uncertainty regarding the toxicity of some organic compounds and arsenosugars towards humans [6].

In plants, arsenic has not been found to have a biological role. Arsenite is believed to be more toxic than arsenate, due to its higher solubility in the soil solution, although some other authors stated that arsenate may entail a major problem for plants. Due to its chemical similarity with phosphate, arsenate can outcompetes with it, replacing phosphate anions in important functions for the plant [36]. The toxicity of organoarsenical compounds have not been enough studied, but some evidences point out that they affect some tissues of the aerial part of the plant, as well as induce some disturbances in the synthesis of proteins [37]. Visual effects of arsenic in plants are evidenced by the growth reduction [38], violet coloration (increased anthocyanin), root discoloration, reduction of root length [39], lack of root ramification, chlorosis in leaves

[40], leaf wilting [36] and even necrosis in the aerial part [41]. Damages in root and leaves make difficult the normal flux of water in the plant. Arsenic also causes oxidative stress [42], nutritional disorders (above all due to the competence with phosphate) [43], photosynthesis inhibition [42], metabolic disorders [44] and genetic alterations [45]. Obviously, when a plantation is located in a contaminated site, yield decreases drastically.

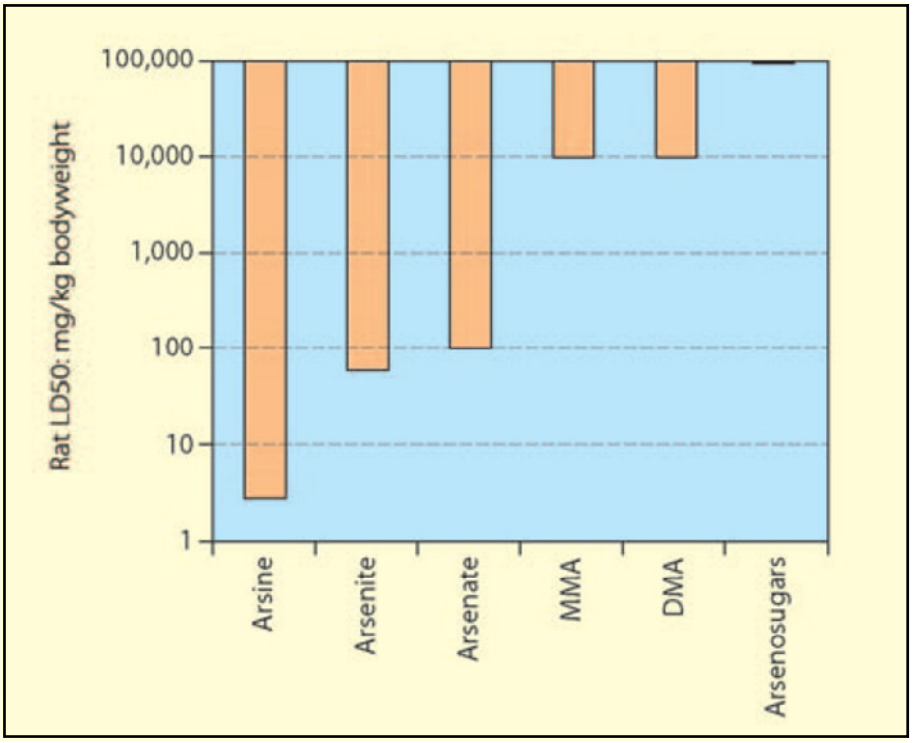

Fig. 1.7. Bar diagram illustrating the relative toxicity of different forms of arsenic. The 'rat LD 50 test' shows the quantity, in milligrams/kilogram bodyweight, of the various arsenic compounds required to kill 50% of a test population of rats [6].

In animals, arsenic has been described as one of the most toxic elements for fish [46]. Acute exposures can result in immediate death because of As-induced increases mucus production, causing suffocation, or direct detrimental effects on the gill epithelium [46]. Chronic exposures can result in the accumulation of the metalloid to toxic levels and the detoxification role of the liver places it at considerable risk [46]. In fish, bizarre morphological alterations, as well as early neoplastic alterations are produced in the liver [46]. The signs of inorganic trivalent arsenite poisoning in birds (mallard, quail, and pheasant) include ataxia, goose-stepping ataxia, asthenia, slowness, jerkiness, falling, hyporeactivity, fluffed feathers, ptosis, huddled position, unkempt appearance, loss of righting reflex, immobility, and tetanic seizures. Signs appeared as soon as 1 hour and mortalities usually after exposure; remission took up

to 1 month [47]. The biochemical effects of arsenic in mammals can be considered rather similar to those in humans.

For humans, the ingestion of elemental arsenic is not considered very harmful, since it is poorly absorbed and easily eliminated unchanged from the human body. However, soluble arsenic compounds pose greater hazard, since they are absorbed from the gastrointestinal tract [48] and eliminated (e.g., As^{5+}, organic arsenic) via kidney [49-52]. Ingested inorganic arsenic is quickly absorbed from the gastrointestinal tract and passes through a series of steps while being metabolized by reduction reactions of pentavalent to trivalent arsenic forms, and methylation to monomethylarsonic acid (MMA) and then to dimethylarsinic acid (DMA). This methylation process has traditionally been considered as the detoxification pathway for inorganic arsenic, resulting in the less toxic MMA and DMA species. Methylation also facilitates the excretion of inorganic arsenic forms from the body. However, the totality of inorganic arsenic is not completely methylated to DMA, as can be shown by the presence of inorganic species as well as MMA and DMA in the urine. The main route of elimination of ingested inorganic arsenic in humans is through urine, accounting for approximately 70% of the intake. The average proportions of arsenic metabolites in urine are about 15–25% inorganic species, 10–15% MMA, and 70–80% DMA [18].

If monomethylarsonic acid (MMA(V)) or dimethylarsonic acid (DMA(V)) are orally administered to humans, they are excreted in the urine largely in the unchanged forms. Only a minor proportion of MMA(V) is converted to DMA(V) and no further metabolization of DMA(V) is observed, although a study reported 4% of trimethylated form of trimethylarsine oxide (TMAO) in urine after administration of DMA(V) [53]. Non-toxic organic arsenic in the forms of arsenobetaine, arsenosugars and arsenolipids can be found in most seafood and seaweed. Arsenobetaine is excreted unchanged in urine but arsenosugars can be metabolized to several compounds including DMA(V) that contribute to the total urinary arsenic [54-56]. Farmer and Johnson (1990) [57] reported that about 40–60% of arsenic may be retained in the body even after exposure cessation and that this may be accumulated in the skin, hair, nails, muscle and small amounts in teeth and bones [52, 58, 59].

Subjects exposed to arsenic show significant inter-individual variation in urinary patterns of arsenic metabolites but insignificant day-to-day intra-individual variation. The inter-individual variation in arsenic methylation can be partly responsible for the variation in susceptibility to arsenic toxicity. Wide inter-ethnic variation and family correlation in urinary arsenic profile suggest a genetic effect on arsenic metabolism.

Women, especially at pregnancy, have higher methylation capacity than their men counterparts, probably due to the effect of estrogens. Children might have better methylation capacity than adults and age shows inconsistent relevance in adults. Smoking and alcohol consumption might be associated with a poorer methylation capacity. Nutritional status is important in the methylation capacity and folate rich diets may facilitate the methylation and excretion of arsenic. Besides, general health conditions and medications might influence the arsenic methylation capacity. The consumption of seafood, seaweed, rice and other food with high arsenic contents and the use of As-containing water in food preparation may also interfere with the presentation of the urinary arsenic profile [52]. In addition, methylation capacity might reduce with increasing dosage of arsenic exposure. It has been hypothesized that beyond a certain threshold of exposure to inorganic arsenic, the methylation capacity would saturate and the toxic effects of inorganic arsenic may increase [55, 60, 61].

The metabolism of arsenic in the human body is not yet fully clarified and there is certain controversy as regards arsenic pathways. However, it seems that some assertions can be affirmed. On one hand, the main organ in charge of arsenic metabolism is the liver [55]. On the other hand, organs can metabolize arsenic by a variety of mechanisms, including methylation, oxidation and reduction reactions, and protein binding [62]. In the pH range of 4 to 10, trivalent arsenic compounds are neutral in charge and pentavalent arsenicals are negatively charged. Therefore, trivalent arsenic species are more prone to cross cell membrane and pentavalent species cannot easily enter the cells at physiologic pH [53]. Inorganic pentavalent arsenate is mostly reduced to trivalent arsenite in the blood stream before entering the cells for further metabolism [53, 63]. At present, it is also known that both arsenite and arsenate can be actively transported into cells via aquaglycoporins and phosphate transporters, respectively [55, 64]. Fig. 1.8 shows two different pathways proposed for arsenic metabolism: the generally accepted classical pathway (Fig. 1A) and a newly proposed pathway (Fig. 1B). In the classical pathway, inorganic trivalent arsenite is a preferential substrate for As(III)-methyltransferase (As3MT, or previously known as cyt19) and the metabolism involves a series of reduction and oxidative methylation reactions, in which the pentavalent arsenic species are formed before the respective trivalent species, and mono- and dimethylated metabolites are sequentially generated (Fig. 1.8 A) [65]. The reduction of MMA(V) to monomethylarsonous acid (MMA(III)) may be the rate-limiting step in the metabolism of inorganic arsenic [53, 66]. As it has been mentioned before, in humans such metabolic process is not complete and inorganic arsenic along with mono- and dimethylated metabolites are excreted in urine. The newly proposed

pathway suggests that trivalent methylated arsenic species might be formed before the respective endproducts of pentavalent species (Fig. 1.8 B) [67]. This newly proposed pathway is compatible with the concept of “oxidation is detoxification of arsenic” [68] because trivalent methylated arsenic species are more toxic than the pentavalent methylated arsenic species and the inorganic species [69]. However, this hypothetical pathway requires further confirmation.

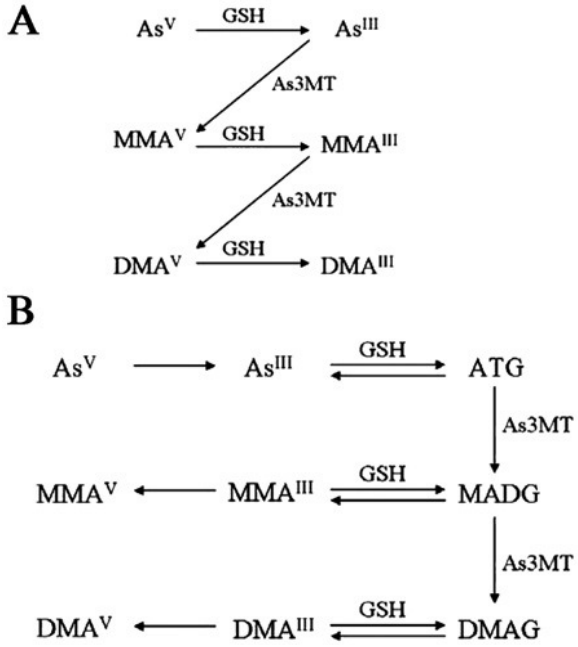

Fig.1.8. The generally accepted classical pathway (A) and the newly proposed pathway (B) for arsenic metabolism. GSH is the electron donor and As3MT uses SAM as methyl donor. As(III), inorganic arsenite; As(V), inorganic arsenate; As3MT, As(+3 oxidation state)-methyltransferase; ATG, arsenic triglutathione; DMAIII, Dimethylarsinous acid; DMAV, Dimethylarsinic acid; DMAG, dimethylarsinic glutathione; GSH, reduced glutathione; MADG, monomethylarsenic diglutathione; MMAIII, Monomethylarsonous acid; MMAV, Monomethylarsonic acid; SAM, S-adenosylmethionine [55].

The toxicity of arsenic for cells arises from its interaction with sulphydryl groups of proteins and enzymes (to denature the proteins and enzymes within the cells) [70, 71] and through an increase of reactive oxygen species in the cells, consequently causing cell damage [72, 73]. Arsenic can interfere with essential enzymatic functions and transcriptional events in cells, leading ultimately to “multitude of multisystemic noncancerous effects that might ensue” [74]. For example, oxidative stress induced by trivalent methylated arsenicals inhibits glutathione (GSH) reductase [75] and thioredoxin reductase [76] with subsequent impairment of cellular protective mechanism against oxidants. While depletion of cellular GSH sensitizes cells to arsenicals and may also contribute to cell transformation [77], thioredoxin depletion affects gene expression due to the fact that it modulates DNA binding activity of some transcriptional factors [78, 79]. Arsenite is known to inhibit more than 200 enzymes in

the body [80] and, because arsenate has a similar structure as phosphate, it can substitute for phosphorus in the body, which can lead to replacement of phosphorus in the bone for many years [81]. Because arsenate is easily hydrolyzed (in the cell), it inhibits subsequent transfer of phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP; the energy currency of the cell) and thus depletes the cell of its energy [82]. Arsine, the most toxic of the arsenicals [83], is known to cause hemolysis of red blood cells, leading to hemolytic anaemia, which is primarily responsible for the development of oliguria renal failure [84]. It has been suggested that arsine interaction with sulphydryl group of proteins and enzymes [85] may be responsible for inhibition of erythrocyte sodium–potassium pump. It is also known that arsenic decreases DNA repair process [86] and, hence, enhances susceptibility to cancer and noncancerous-related diseases [52]. Fig. 1.9 summarizes some of the most important biochemical effects provoked by arsenic.

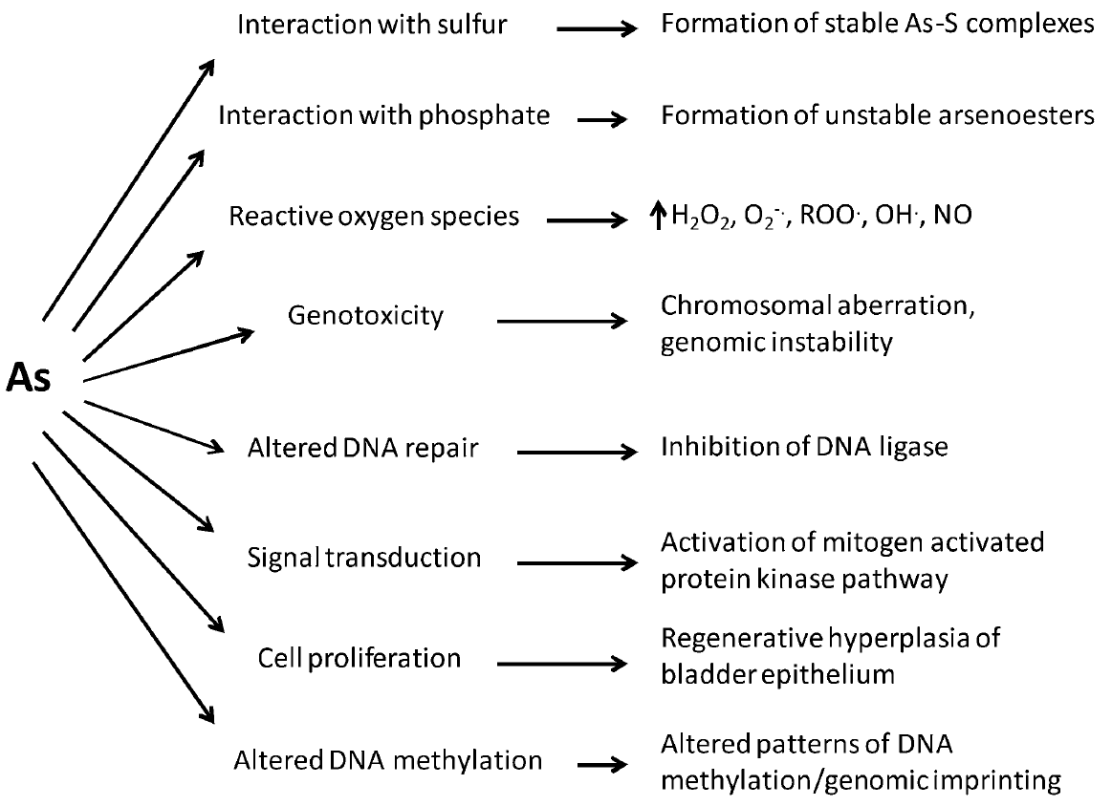

Fig. 1.9. Modes of action of arsenic and their consequent biochemical effects [87].

1.2.4 Health risks and effects to humans

The health effects that arsenic provokes in humans derive from its toxicological properties and its interactions with the different compartments in the human body, as it has been explained in the previous section.

The great concerns over arsenic and human health today centre upon the long-term ingestion of this element, chiefly in drinking water, although there are other access routes for arsenic to human body, such as the skin and the respiratory and the digestive systems. Due to the presence of arsenic in the atmosphere and in soils, sediments and rocks, arsenic can be uptaken by plants and animals and finally reach the foodstuff chain. Fig. 1.10 illustrates the interactions between the different compartments of the environment and the possible pathways for arsenic to humans.

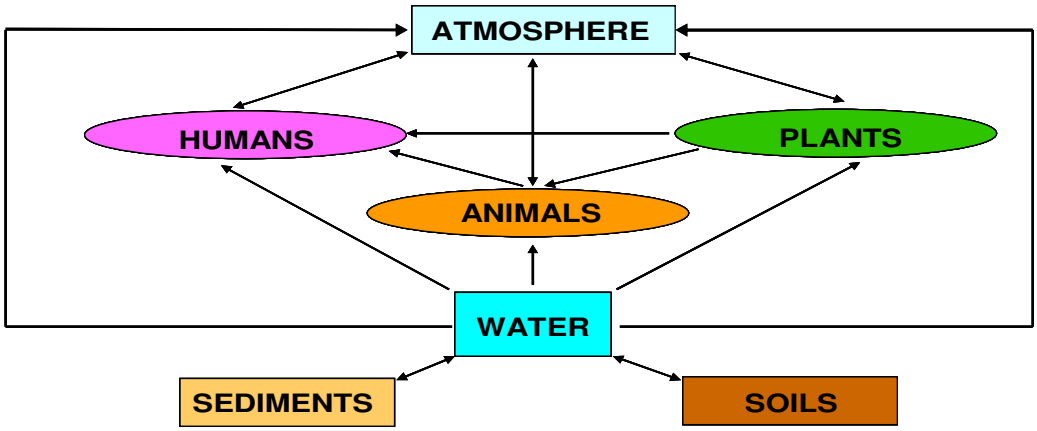

Fig. 1.10. Interaction between living organisms and environment.

Long-term exposure to arsenic results in chronic arsenic poisoning (arsenicosis). This has been reported to occur in people who live in endemic areas with high arsenic concentrations in drinking water or in burning coal [88-93]. Arsenicosis has also been reported in people with occupational exposure to arsenic [94]. In several parts of the world, arsenic-induced diseases are a significant public health problem.

Arsenic is one of the few substances shown to cause cancer in humans through consumption of drinking-water [95-98]. There is overwhelming evidence from epidemiological studies that consumption of elevated levels of arsenic through drinking-water is causally related to the development of skin cancer (Fig. 1.11), and increases the risk of cancers of the bladder, lung, kidney, liver, colon and prostate [95-99]. Thus, inorganic arsenic compounds are classified by IARC in Group 1 (carcinogenic to humans) on the basis of sufficient evidence for carcinogenicity in humans and limited evidence for carcinogenicity in animals [6, 36, 100, 101]. In addition to cancer related problems arsenic exposure entails gastrointestinal, skin, and hepatic symptoms; disturbances of cardiovascular, respiratory, nervous, and immunological system functions [102]. However, recent studies suggest that the development of human chronic diseases associated with arsenic is not only determined by the dosage of

exposure, but also by the individual methylation capacity [103, 104] and the metabolic patterns [65, 105-107].

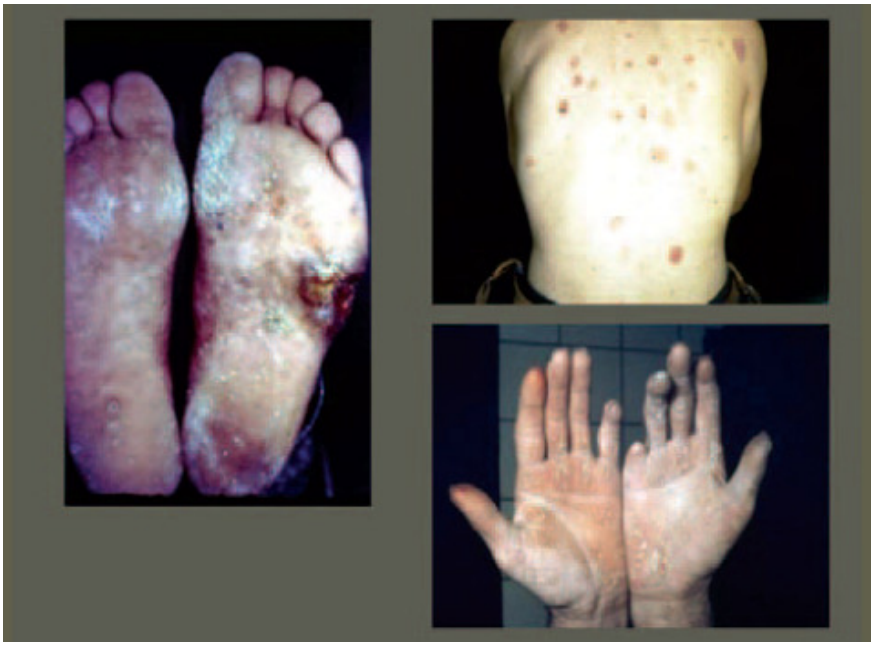

Fig. 1.11. Skin keratoses, amputations, and skin cancer provoked by arsenic exposure.

1.3 ARSENIC IN THE ENVIRONMENT

1.3.1 Applications and uses of arsenic

Although arsenic had multiple applications in the past, nowadays its use has greatly declined partly because of a much fuller appreciation of its toxicity at even low concentration levels.

The main use of metallic arsenic is for alloying with copper and especially lead. Arsenic imparts to copper a very fine white colour, and makes it very hard and brittle. Before German silver was known, those alloys were sometimes used for the manufacture of such cast articles. When exposed to the air, they soon lose their whiteness and take on a brownish shade. On account of this, as well as the poisonous character of the arsenic, it is very little used at the present time. Lead components in automotive batteries are strengthened by the presence of a few percent of arsenic. Gallium arsenide is an important semiconductor material used in integrated circuits, much faster than those made in silicon, and so can be used in lased diodes and LEDs to directly convert electricity into light [108].

Historically, it was also used in pigments to colour wallpapers, paints and ceramics, and in glass manufacture [6].

Arsenic has a special place in the history of modern medicine. In 1910, Paul Ehrlich invented a drug called salvarsan (whose chemical name is arsphenamine) as a remedy for syphilis. Thomas Fowler started to employ arsenic trioxide in the treatment of cancer.

The harmful nature of arsenic to insects, bacteria and fungi led to use it as a wood and hide preservative, insecticide and rodenticide, and as agricultural pesticides and weedkillers [109].

Arsenic, as the chemical compound roxarsone, is still used as additives in animals feed for disease prevention and growth stimulation. [6]

Arsenic has also had military uses. After World War I, the United States built up a stockpile of 20000 tons of lewisite ($ClCH{=}CHAsCl_2$), a chemical weapon that is a vesicant (blister agent) and lung irritant. The stockpile was neutralized with bleach and dumped into the Gulf of Mexico after the 1950s. During the Vietnam War, the United States used Agent Blue, a mixture of sodium cacodylate and its acid form, as one of the rainbows herbicides to deprive the Vietnamese of valuable crops.

1.3.2 Arsenic sources and natural forms of occurrence

Arsenic is an element naturally present in the environment. Long before man's activities had any effect on the balance of nature, arsenic was ubiquitously distributed throughout earth's crust, soil, sediments, water, air and living organisms [110]. The major occurrence of arsenic is due to the mineralogical and geological characteristics of the areas and most environmental problems associated to arsenic are the result of mobilization under natural conditions, as volcanic activity.

However, man has had an important additional impact through mining activity and ore processing (Fig. 1.12), combustion of fossil fuels (Fig. 1.13), the use of arsenical pesticides, herbicides and crop desiccants and all the uses of arsenic previously mentioned [14, 111, 112] (section 1.3.1). The worldwide arsenic production has been estimated between 75 and $100 \cdot 10^3$ tons per year [111]. Although the use of arsenical products has decreased significantly in the last few decades, its environmental impact, at least locally, will remain for some years [113].

In most rocks it ranges from 0.5 to 2.5 $mg \cdot kg^{-1}$ [114], though higher concentrations were found in finer grained argillaceous sediments and phosphorites. Arsenic is concentrated in some reducing marine sediments, which may contain up to 3000 $mg \cdot kg^{-1}$. Arsenic may be co-precipitated with iron hydroxides and sulphides in sedimentary rocks. Iron deposits, sedimentary iron ores and manganese nodules can be rich in arsenic [110]. Arsenic contents in various geochemical materials are listed in Table 1.1

Fig. 1.12. Ore processing.

Fig. 1.13. Fossil fuel burning.

Arsenic naturally occurs in over 200 different mineral forms, of which approximately 60% are arsenates, 20% sulphides and sulphosalts and the remaining 20% includes arsenides, arsenites, oxides, silicates and elemental arsenic (As) [115]. A list of some of the most common arsenic minerals is given in Table 1.2. Most of them are ore minerals or their alteration products. However, these minerals are relatively rare in the natural environment. The highest concentrations of those minerals occur in mineralized areas and they are found in close association with the transition metals as well as Cd, Pb, Ag, Au, Sb, P, W and Mo [113]. Although arsenopyrite (FeAsS) is believed to be the most abundant arsenic ore mineral, arsenian ('As-rich') pyrite ($Fe(S,As)_2$) is probably the most important source of arsenic in ore zones [116]. It is generally assumed that arsenopyrite, together with the other dominant arsenic sulphide minerals such as realgar and orpiment, are only formed under high temperature conditions in the earth's crust. However, authigenic arsenopyrite has been reported in sediments [117] and orpiment can been formed by microbial precipitation [118]. Where arsenopyrite is present in sulphide ores associated with sediment-hosted Au deposits, it tends to be the earliest-formed mineral, derived from hydrothermal solutions and formed at typically temperatures of 100 ºC or more. This is followed by the formation of rarer native arsenic and thereafter arsenian pyrite. Realgar and orpiment generally are formed later still. This paragenetic sequence is often reflected by zonation within

sulphide minerals, with arsenopyrite cores zoning out to arsenian pyrite and realgar-orpiment rims. Oxides and sulphates are formed at the latest stages of ore mineralization [113, 119].

Table 1.1. Arsenic concentrations in various terrestrial materials [110].

Materials	Arsenic (mg kg^{-1})
Igneous [120]	
Acidic	
Rhyolite (extrusive)	3.2–5.4
Granite (intrusive)	0.18–15
Intermediate	
Latite, andesite, trachyte (extrusive)	0.5–5.8
Diorite, granodiorite, syenite (intrusive)	0.09–13.4
Basic	
Basalt (extrusive)	0.18–113
Gabbro (intrusive)	0.06–28
Ultrabasic	
Peridotite, dunite, serpentinite	0.3–15.8
Metamorphic rocks [120]	
Quartzite	2.2–7.6
Slate/phyllite	0.5–143
Schist/gneiss	0.0–18.5
Sedimentary rocks [115,121,122]	
Marine	
Shale/claystone (nearshore)	4.0–25
Shale/claystone (offshore)	3.0–490
Carbonates	0.1–20.1
Phosphorites	0.4–188
Sandstone	0.6–9
Non-marine	
Shales	3.0–12
Claystone	3.0–10
Recent sediments (marine)	
Muds [115]	3.2–60
Clays [115]	4.0–20
Carbonate [123]	<1.0
Stream/river [124]	5.0–4000 (mineralized area)

Table 1.2. Major arsenic minerals occurring in nature [125].

Mineral	Composition	Occurrence
Native arsenic	As	Hydrothermal veins
Proustite	Ag_3AsS_3	Generally one of the late Ag minerals in the sequence of primary deposition
Rammelsbergite	$NiAs_2$	Commonly in mesothermal vein deposits
Safflorite	$(Co,Fe)As_2$	Generally in mesothermal vein deposits
Seligmannite	$PbCuAsS_3$	Occurs in hydrothermal veins
Smaltite	$CoAs_2$	–
Niccolite	NiAs	Vein deposits and norites
Realgar	AsS	Vein deposits, often associated with orpiment, clays and limestones, also deposits from hot springs
Orpiment	As_2S_3	Hydrothermal veins, hot springs, volcanic sublimation product
Cobaltite	CoAsS	High-temperature deposits, metamorphic rocks
Arsenopyrite	FeAsS	The most abundant As mineral, dominantly mineral veins
Tennantite	$(Cu,Fe)_{12}As_4S_{13}$	Hydrothermal veins
Enargite	Cu_3AsS_4	Hydrothermal veins
Arsenolite	As_2O_3	Secondary mineral formed by oxidation of arsenopyrite, native arsenic and other As minerals
Claudetite	As_2O_3	Secondary mineral formed by oxidation of realgar, arsenopyrite and other As minerals
Scorodite	$FeAsO_4{\cdot}2H_2O$	Secondary mineral
Annabergite	$(Ni,Co)_3(AsO_4)_2{\cdot}8H_2O$	Secondary mineral
Hoernesite	$Mg_3(AsO_4)_2{\cdot}8H_2O$	Secondary mineral, smelter wastes
Haematolite	$(Mn,Mg)_4Al(AsO_4)(OH)_8$	–
Conichalcite	$CaCu(AsO_4)(OH)$	Secondary mineral
Adamite	$Zn_2(OH)(AsO_4)$	Secondary mineral
Domeykite	Cu_3As	Found in vein and replacement deposits formed at moderate temperatures
Loellingite	$FeAs_2$	Found in mesothermal vein deposits
Pharmacosiderite	$Fe_3(AsO_4)_2(OH)_3{\cdot}5H_2O$	Oxidation product of arsenopyrite and other As minerals

1.3.3 Environmental transport and distribution

Arsenic can be found in all the compartments of the environment. Fig. 1.14 shows the sources and sinks of this element in the geosphere.

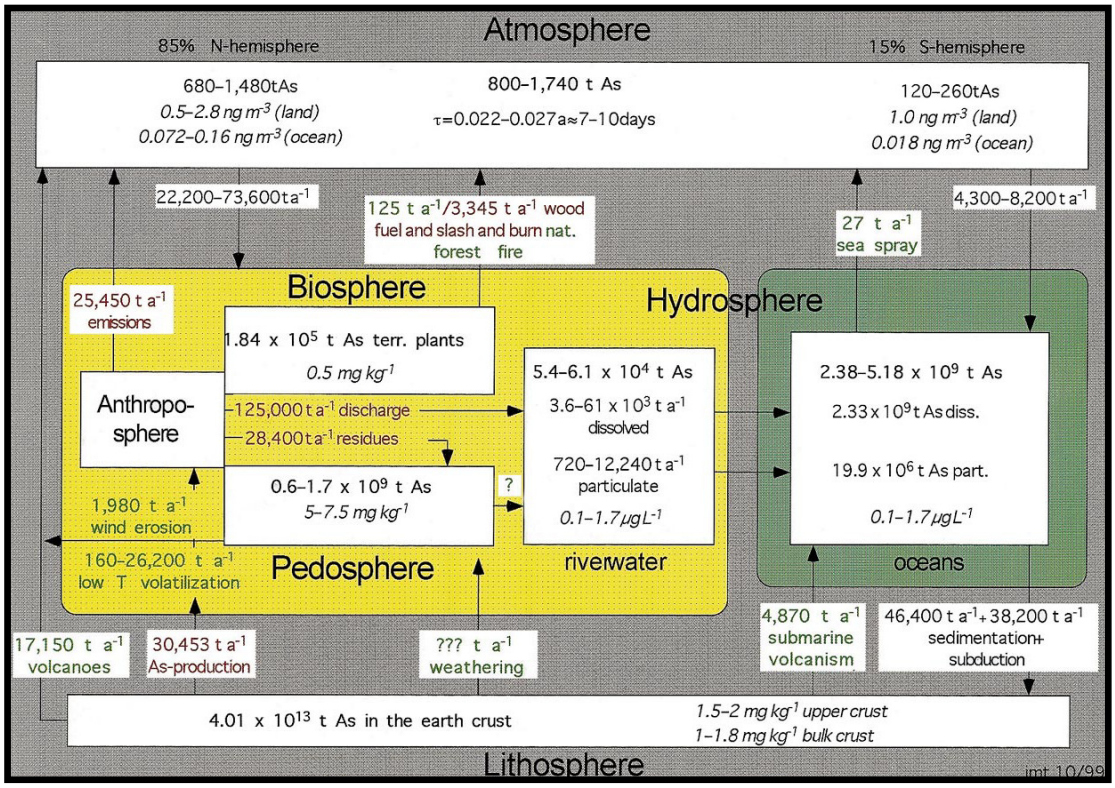

Fig. 1.14. Global arsenic cycle. Fluxes and reservoir sizes in the different parts of the geosphere [126].

Arsenic is emitted into the atmosphere by high-temperature processes such as coal-fired power generation plants, burning vegetation and volcanism. Natural low-temperature biomethylation and reduction to arsines also release arsenic into the atmosphere. Arsenic is released into the atmosphere primarily as As_2O_3 and it exists mainly adsorbed on particulate matter. These particles are dispersed by the wind and are returned to the earth's surface by wet or dry deposition. Arsines, released from microbial sources in soils or sediments, undergo oxidation in the atmosphere, reconverting the arsenic to non-volatile forms, which settle back to the ground. Dissolved forms of arsenic in the water column include arsenate, arsenite, MMA and DMA. Weathered rock and soil may be transported by wind or water erosion. Many arsenic compounds tend to adsorb to soils, and leaching usually results in transportation over only short distances in soil [127].

Three major ways of arsenic biotransformation have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and

methylation of arsenic, and the biosynthesis of organoarsenic compounds. Compounds formed from these processes are biogeochemically cycled [127].

1.3.4 Arsenic in soils and sediments

1.3.4.1 Arsenic abundance and legislation about contaminated soils

The presence of arsenic in soils can be related to the geological substratum, which implies that a wide range of arsenic concentrations can be found in uncontaminated soils, depending on the geographic regions [110]. Arsenic is present in soils at higher concentrations than those in rocks [128]. Regarding soils, sandy ones and those derived from granites, usually contain lower arsenic concentrations than alluvial and organic soils [114]. Arsenic concentration in uncontaminated soils usually range 1–40 $mg \cdot kg^{-1}$, and the geochemical background reported is between 5 and 10 $mg \cdot kg^{-1}$ [126, 129].

Sediments in aquatic systems often have higher arsenic concentrations than those of the water [130]. The natural values reported for uncontaminated sediments are below 10 $mg \cdot Kg^{-1}$ [131] and also vary considerably all over the world.

Anthropogenic activities are often responsible for a higher accumulation of arsenic in soils and sediments [132]. For instance, agricultural practices may be a significant source of arsenic, due to its contents in pesticides, fertilizers, sludge and manure. Thus, increasing contents of arsenic in agricultural soils have recently become a real problem. This is especially emphasized in the case of paddy soils that are highly modified by anthropogenic activities. Paddy soils are often contaminated by fertilizers and organic chemicals, especially in those areas where rapid economic development is needed [133]. The irrigation with As-loaded groundwater increases its level in soils [134]. The arsenic contents in this kind of soils of Bangladesh range between 2 and 80 $mg \cdot kg^{-1}$ [135], substantially exceeding the guideline limit of 50 $mg \cdot kg^{-1}$ established for agricultural soils [136]. In Spain, total arsenic contents in agricultural soils irrigated with As-rich groundwater were also found to exceed the arsenic content in the considered control areas [137].

Mining works, ore processing and waste discharge of metal processing plants can led to the release of huge amounts of arsenic that can be fixed by soils and sediments of these areas. Therefore, arsenic concentrations in tailings piles and soils affected by tailings can reach up to several thousand $mg \cdot kg^{-1}$, as those values reported

by Nagorski et al. (1999) in tailing-contaminated soils from Montana (up to 1100 $mg \cdot kg^{-1}$) [138], or those reported by Kavanagh et al. (1997) in tailings-contaminated soils in UK (up to 52600 $mg \cdot kg^{-1}$) [139]. In Spain, up to 150 $mg \cdot kg^{-1}$ of arsenic have been found in soils from mining areas in the province of Salamanca [140], more than 2500 $mg \cdot kg^{-1}$ in the surrounding soils of La Parrilla mine in Cáceres [141], greater concentrations than 11000 $mg \cdot kg^{-1}$ in the surroundings of mine sites in Asturias [142] and arsenic concentrations exceeding 67000 $mg \cdot kg^{-1}$ for spoil heap samples from a mine dedicated to arsenic exploitation in the province of León [143].

Although the legislation governing arsenic contamination in waters is well defined, the regulatory goals for remediation of contaminated soils greatly vary among countries and even locally [144]. Thus, the USEPA (United States Environmental Protection Agency), after having performed a conservative risk analysis [145] stated that arsenic concentrations in soil can reach 40 $mg \cdot kg^{-1}$ without a hazard to exposed organisms [146].

The European Union is paying special attention to the regulation of contaminated sites developing a strategy on soil protection, included in the 6th Action Plan [147], but some working is still being carried out for the establishment of the possible contaminants in soils. At present, there is no clear legislation as regards this. There only exists the ongoing pretension that the Member States make an inventory of their polluted soils, including measurements of the concentrations of the pollutants. If these levels are decided to pose a significant risk to human health or the environment, then an in-situ evaluation of the potential hazard should be performed [148]. In this sense, several Member States are developing or updating national strategies for the evaluation and control of contaminated soils. For instance, Netherlands have set the action threshold in 76 $mg \cdot kg^{-1}$ of arsenic for all kind of soils [149]. UK makes a differentiation among residential or agricultural soils and soils for commercial or industrial purposes, establishing the limit concentration in 20 $mg \cdot kg^{-1}$ for the former case whereas for the latter situation this value can reach up to 500 $mg \cdot kg^{-1}$ [150]. Belgium allows up to 200 $mg \cdot kg^{-1}$ for recreational areas and 110 $mg \cdot kg^{-1}$ for residential soils [111].

Nonetheless, the concept of contaminated soil is changing. The traditional criterion considered a soil as contaminated if the amount of certain hazardous substance exceeded an established limit of concentration, from which it could represent a threat to humans or the environment. Nowadays, a soil can be considered

polluted if the presence of certain chemical compounds can alter its functionality or its quality.

In Spain, the last proposal as regards soils contamination is the “Real Decreto 9/2005”. Obviously, it is fully integrated within the European legal framework. Following the European policy on chemicals, the decision is risk-oriented instead of hazard-oriented [151]. The overall approach is an adaptation of generic risk assessment procedures such as those used in Europe (SSC 2003) [152] or elsewhere (USEPA 1989, 1998) [145, 153]. Whenever possible, the European approaches for estimating the risk of industrial chemicals (ECB 2003) have been used [154], with the required adaptation for moving from a generic substance-based risk assessment methodology to site-specific risk assessment. For Human Health risks, the degree of permissibility depends on the use of the soil. Three different possibilities are considered: exclusively, industrial soils, residential areas (where industrial activities are also allowed), and soils for which all potential uses are allowed (including recreational and agricultural activities). Table 1.3 presents the covered exposure routes for each soil use. Obviously, the level of protection increases from industrial to all-use sites. For the protection of ecosystems, the approach is slightly different. The soil uses are not considered relevant, and the ecological receptors requiring protection should instead be established. Three main groups of ecological receptors are considered: Soil dwelling organisms including plants, aquatic ecosystems, and terrestrial vertebrates. The organisms and exposure routes covering each receptor are presented in Table 1.4. The maximum soil concentrations resulting in acceptable risk under these worst-case estimations are named Generic Reference Levels (Nivel Genérico de Referencia or NGRs according to the Spanish spelling) and if any of these generic reference levels is exceeded, a site-specific risk assessment is required. The Spanish “Real Decreto 9/2005” establishes that a soil is contaminated by a certain pollutant if its concentration exceeds 100 times the NGR level, which for substances with carcinogenic effects (genotoxic) is that which makes that the probability of suffer from cancer entails an additional case per 100,000 exposed human beings. However, the regulation does not cover the development of generic reference limits for metals; transferring this responsibility to the regional governments. As an alternative, the regulation allows the definition of site-specific reference levels as the mean plus twice the standard deviation of background levels measured in the surrounding area. Some regions in Spain have already calculated their “generic reference levels”, since they are the bodies responsible for the declaration of a soil as contaminated. For instance, in the region of Madrid, the NGR for arsenic in soils for industrial purposes has been considered 40

mg·kg^{-1}, whereas for urban soils and soils devoted to other uses this value decreases to 24 mg·kg^{-1} [155].

Table 1.3. Exposure routes covered in the human health risk assessment for contaminated soils according to the Spanish "Real Decreto 9/2005" [151].

Soil use	Exposure routes
Industrial	Vapour inhalation Particle inhalation Soil ingestion
Residential (including industrial)	Vapour inhalation Particle inhalation Soil ingestion Dermal contact
All uses	Vapour inhalation Particle inhalation Soil ingestion Dermal contact Consumption of food items produced in the soil

Table 1.4. Organisms and exposure routes used for setting generic reference limits for each ecological receptor, according to the Spanish "Real Decreto 9/2005" [151].

Ecological receptor	Organisms covered	Exposure routes
Soil organisms	Soil microbial functions Terrestrial plants Soil invertebrates	Soil
Aquatic systems	Fish Aquatic invertebrates Algae and aquatic plants	Drainage of soil pore water
Terrestrial vertebrates	Mammals Birds Other vertebrates	Consumption of contaminated plants, invertebrates and vertebrates

Fitz and Wenzel (2002) described that in the European Union 1.4 million of soils could be contaminated by metals, metalloids and/or organic compounds (taking into account that at the time of the study only 15 of the current 27 states composed the European Union). Furthermore, these authors estimated that in the catalogue of contaminated soils of U.S.A. around 41% of the soils were contaminated with arsenic whereas in Australia there are 10,000 soils contaminated by this element [112].

An important aspect to consider in the study of arsenic occurrence in soils is the heterogeneity that it presents, resulting in areas with high arsenic concentrations adjacent to others less affected [156]. Especially when there is a specific source of arsenic pollution, the degree of contamination is usually reduced with the distance to the focus. Some authors have reported higher arsenic levels in upper horizons of soils [111, 157], indicating that an episode of contamination had occurred before the soil genesis.

1.3.4.2 Arsenic geochemistry in soils

The geochemistry of arsenic in soils and sediments is very complex, due to the huge number of factors affecting its dynamic, transport, reactivity, mobility and availability. The behaviour of this metalloid mainly depends on: i) the chemical species that it presents ii) the soil solution-solid partitioning and iii) the kind of associations with the solid phases. Thus, in this section we will examined: 1) the factors controlling the presence of this element in the soil solution, 2) the different types of arsenic retention in the soil and its relative availability as a function of the strength of the binding, and 3) the most important parameters influencing its form of occurrence and its potential mobility. At the end of the section, a critical discussion based on sequential extraction procedures from the literature as a tool for the assessment of the availability of an element in the environment will be performed.

Despite the chemical speciation of arsenic is highly dependent on several factors, it seems general that it usually occurs as oxyanions (in the oxidation states (III) and (V)) in soils and sediments due to its great affinity for oxygen [14, 158]. Arsenite is the predominant form under reducing conditions, such as regularly flooded soils, whereas arsenate species dominate in oxygen-rich environments and well-drained soils ($H_2AsO_4^-$ in acidic soils and $HAsO_4^{2-}$ in alkaline ones) [33, 159].

It is well known that the toxicity of an element depends on its chemical form and therefore on its relative mobility and availability. As(V) is less soluble and less mobile than As(III) and consequently, less toxic [33, 160].

The solubility of the chemical species present in soils is a parameter of paramount importance to predict the concentration of a pollutant in the soil solution. Table 1.5 shows the thermodynamic equilibrium constants for arsenic species. These data refer to 25ºC, one atmosphere pressure and zero ionic strength. It must be taken into account that these are idealized conditions and different from those found in soil solutions. Nonetheless, under the foregoing limitations, thermodynamic solubility calculations can predict trends and guidelines for the chemical forms of arsenic in solution.

For arsenic, the typical concentrations in the soil solution in aerobic conditions have been reported below 53 nM for uncontaminated soils, whereas they reach up to 2.3 µM in contaminated soils [161]. For flooded soils, the arsenic concentration in the soil solution ranges from 0.01 to 3 µM [162].

Table 1.5. Equilibrium reactions and constants (log K^0) of arsenic species at standard conditions [163].

No.	Chemical reactions	Log K^0
A: Solid Species of As		
1	$As_2O_3(c,arsenolite) + 5H_2O \Longleftrightarrow 2HAsO_4^{2-} + 8H^+ + 4e^-$	–58.68
2	$As_2O_3(c,claudenite) + 5H_2O \Longleftrightarrow 2HAsO_4^{2-} + 8H^+ + 4e^-$	–58.78
3	$As_2O_5(c) + 3H_2O \Longleftrightarrow 2HAsO_4^{2-} + 4H^+$	–11.63
4	$AsS(c,realger) + 8H_2O \Longleftrightarrow HAsO_4^{2-} + SO_4^{2-} + 15H^+ + 11e^-$	–83.13
5	$As_2S_2(c,orpiment) + 16H_2O \Longleftrightarrow 2HAsO_4^{2-} + 2SO_4^{2-} + 30H^+ + 22e^-$	–180.43
6	$As_2S_3(c) + 20H_2O \Longleftrightarrow 2HAsO_4^{2-} + 3SO_4^{2-} + 30H^+ + 33e^-$	–219.14
7	$AlAsO_4(c) + H^+ \Longleftrightarrow Al^{3+} + HAsO_4^{2-}$	–4.70
8	$Ba_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Ba^{2+} + 2HAsO_4^{2-}$	–26.50
9	$Ca_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Ca^{2+} + 2HAsO_4^{2-}$	–1.91
10	$Cd_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Cd^{2+} + 2HAsO_4^{2-}$	–8.97
11	$Cu_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Cu^{2+} + 2HAsO_4^{2-}$	–14.97
12	$FeAsO_4(c) + H^+ \Longleftrightarrow Fe^{3+} + HAsO_4^{2-}$	–9.45
13	$Fe_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Fe^{2+} + 2HAsO_4^{2-}$	–18
14	$Mn_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Mn^{2+} + 2HAsO_4^{2-}$	–8.51
15	$Ni_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Ni^{2+} + 2HAsO_4^{2-}$	–2.21
16	$Pb_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Pb^{2+} + 2HAsO_4^{2-}$	–9.07
17	$Zn_3(AsO_4)_2(c) + 2H^+ \Longleftrightarrow 3Zn^{2+} + 2HAsO_4^{2-}$	–8.20
B: Solution Species of As		
18	$AsO_2^- + 2H_2O \Longleftrightarrow HAsO_4^{2-} + 3H^+ + 2e^-$	–19.31
19	$HAsO_2^0 + 2H_2O \Longleftrightarrow HAsO_4^{2-} + 4H^+ + 2e^-$	–28.60
20	$AsO_2^- + H^+ \Longleftrightarrow HAsO_2^0$	–9.29
21	$AsO_3^{3-} + H_2O \Longleftrightarrow HAsO_4^{2-} + H^+ + 2e^-$	–6.13
22	$HAsO_3^{2-} + H_2O \Longleftrightarrow HAsO_4^{2-} + 2H^+ + 2e^-$	–7.27
23	$H_2AsO_3^- + H_2O \Longleftrightarrow HAsO_4^{2-} + 3H^+ + 2e^-$	–19.49
24	$H_3AsO_3^0 + H_2O \Longleftrightarrow HAsO_4^{2-} + 4H^+ + 2e^-$	–28.63
25	$AsO_3^{3-} + H^+ \Longleftrightarrow HAsO_3^{2-}$	–12.71
26	$AsO_4^{3-} + H^+ \Longleftrightarrow HAsO_4^{2-}$	12.19
27	$HAsO_4^{2-} + H^+ \Longleftrightarrow H_2AsO_4^-$	6.94
28	$H_2AsO_4^- + H^+ \Longleftrightarrow H_3AsO_4^0$	2.24
C: Organic compounds[a]		
29	$CH_3{\cdot}AsO_3^{2-} + H^+ \Longleftrightarrow CH_3{\cdot}HAsO_3^-$	8.24
30	$CH_3{\cdot}HAsO_3^- + H^+ \Longleftrightarrow CH_3{\cdot}H_2AsO_3^0$	3.61

The soluble fraction of an element is in equilibrium with its concentration in the solid phases. Soil is a heterogeneous system and an array of arsenic reactions with different soil components can occur, either individually or simultaneously. The Fig. 1.15 illustrates the different ways of arsenic retention in the solid phases and the ways of occurrence in the soil solution.

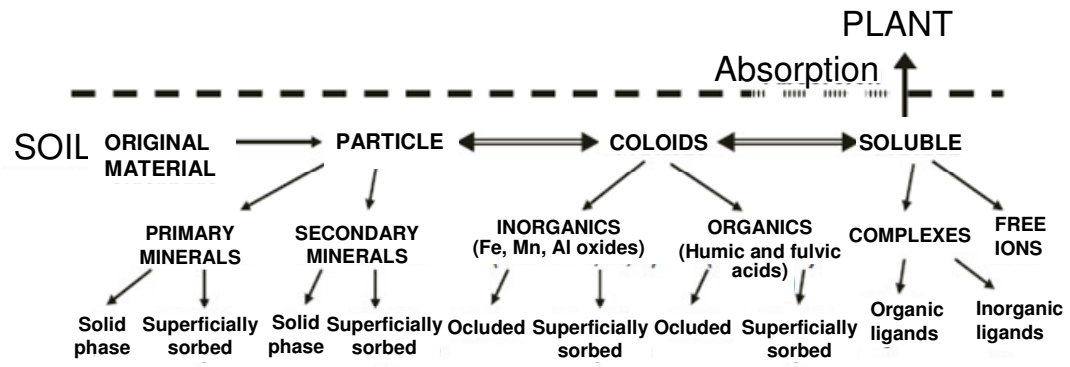

Fig. 1.15. Factors influencing the concentration of an element in the soil solution. Modified from Menguel y Kirkby (2001) [164]; Juárez et al. (2006) [165].

One of the most commonly reported, and perhaps the first reaction to occur in soils is adsorption on soil colloids (clays, oxides or hydroxides surfaces of Al, Fe and Mn, calcium carbonates and organic matter). Irrespective of the mechanisms of charge development, soil colloids carry substantial pH-dependent charge. Many factors, such as type, crystallinity, the chemical composition of the colloids, pH of the surrounding media, type of impurities present in the colloid, etc. affect the isoelectric point of pH dependent charge on soil colloids [163]. The pH dependence of some inorganic and organic substances to retain arsenic will be further discussed. Nevertheless, adsorption reactions are very complex and confusing, and the differentiation between a purely adsorption process and a chemical reaction often results very difficult. In fact, both processes may occur simultaneously very often, which is commonly known as “chemisorption”.

Direct precipitation of arsenic solid phases may not occur except in the arsenic contaminated soils. It has been reported that the weathering of primary arsenic minerals present in arsenic contaminated soils leads to the formation of other secondary forms which are thermodynamically more stable. This fact can occur either through the adsorption of arsenic onto colloid surfaces, or by direct precipitation of arsenic solid phases [163, 166]. In the first case, secondary precipitation of arsenic compounds may occur in soil colloid surfaces subsequently to its adsorption. In the secondary precipitation, the first step would probably be the adsorption of dissolved arsenic species onto soil colloidal surfaces as shown in Fig. 1.16. The adsorbed arsenic ions would gradually and continuously move inside the hydrated mineral layer up to reach a level to precipitate as an arsenic solid phase [163]. Arsenates constitute the main arsenic secondary minerals present in soils and sediments [167, 168].

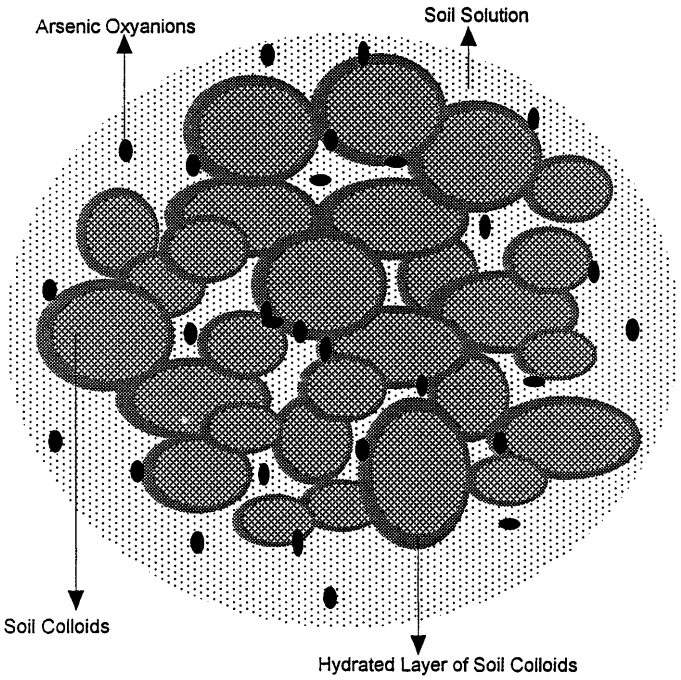

Fig. 1.16. Chemisorption of arsenic oxyanions on soil colloids [163].

As it has been mentioned before, arsenic is retained in the soil or sediment by different solid phases and the strength of the binding is largely dependent on the considered pool. Hence, the availability of this element depends on the type of association. Table 1.6 shows the different ways of retention of an element in the soil and its relative availability.

Table 1.6. Different ways or retention of an element by the soil. Modifed from Cala (1995) and Van Herreweghe et al. (2003) [33].

Way of As occurrence in the soil	Relative availability
Ion in the soil solution	Readily soluble
Occupying exchangeable sites as diffuse ion or as outer-sphere complexes	Easily available
Specifically adsorbed as inner-sphere complexes	Relatively available
Bound to organic matter	Little available
(Co)precipitated as pure or mixed solids	Available after chemical alteration
Present in the structure of primary or secondary minerals	Drastic weathering

The mobility and availability of arsenic in soils and sediments is conditional upon the characteristics of the site. Hence, it is subjected to many factors, as chemical parameters (pH, Eh, presence of some oxy(hydroxides), organic matter, phosphorus…), environmental factors (climate, orography…) and biological processes (presence of microorganisms that can alter the original speciation…). All of them

contribute to the dynamics of arsenic in soils and sediments. Alteration of the environmental conditions of the site can cause variability in arsenic speciation and solubility of arsenic compounds, can affect the dissolution or precipitation of different minerals that may provide sorption sites for arsenic, and can change the surface charge on different solid phases [163]. The effects exerted by the more important factors controlling arsenic mobility are detailed below.

pH

Soil pH is an important factor determining arsenic mobilization in soils and sediments because this parameter governs the adsorption capacity of the soil constituents.

Adsorption has been stated as the main mechanism of arsenic scavenging in soils and sediments. This phenomenon takes place when the surface of the solid phase presents an opposite charge to the arsenic species. It is assumed that arsenic generally appears as oxyanions in soils and sediments. Hence, the retention will be effective in the range of pH for which the mineral surface is positively charged. As mentioned before, soil is a complex system where a great variety of constituents coexist. Depending on their point of zero charge (PZC), these phases will be positively or negatively charged at different pH values. Fig. 1.17 shows the charge distribution of the most important phases retaining arsenic as a function of pH. The darker parts indicate the ranges of pH where arsenic adsorption likely occurs. The PZC for Mn oxides/hydroxides is about 4, which implies that those minerals only may adsorb arsenic at acid pH. This value is approximately 6 for Al oxides/hydroxides, meaning that those mineral phases only may be effective in the arsenic retention also at acid pH values. The same happens with the clay materials, whose PZC is around 5. Consequently, it seems that these phases would play a limited role in the arsenic adsorption above soil pH 3, 5 and 4 respectively. However, Fe oxides/hydroxides present positively charged surface at pH values< 8. This, together with the high specific surface that these minerals present (30-300 $m^2 \cdot g^{-1}$) make the Fe oxides/hydroxides excellent arsenic scavengers for most soils, since they usually exhibit pH below 8. Although clays usually present high specific surface, their PZC indicates that their adsorption capacity is limited only to very acidic soils. As regards carbonates, they would adsorb arsenic only between pH 7 and 9 [163].

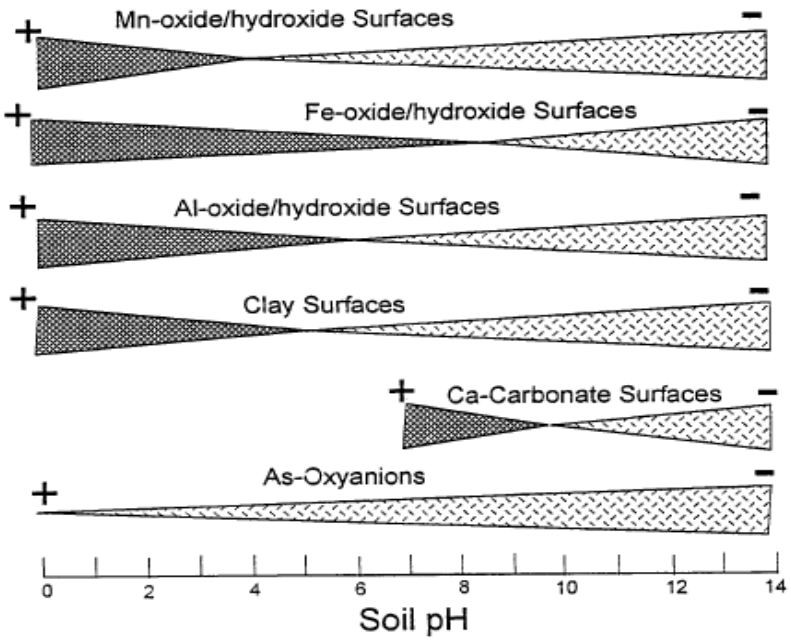

Fig. 1.17. General charge distribution in some important minerals retaining arsenic [163].

The total charge of the soil will be the sum of the charges of the different constituents. An increase of soil pH will deprotonate the mineral surfaces and therefore they will be negatively charged, which provokes the desorption of arsenic oxyanions. Hence, contrary to what occurs with most trace metals, as pH increases, especially above pH 8.5, arsenic is desorbed from the mineral surfaces, thereby increasing its concentration in solution [163, 169].

Some cations, because of their positive charge, may also promote the adsorption of negatively charged arsenate [170]. Calcium and magnesium are probably the most important cations in this respect because of their abundance in most natural waters and their divalent charge. Fe(II) may be important in reduced waters and Al in acidic waters. Silica also exerts a control on the sorption of arsenic [171].

The predominance of As(III) or As(V) in the solution is also consequence of the adsorption and desorption processes and pH. As mentioned before, iron oxyhydroxides are the main minerals adsorbing arsenic, and it has been established that arsenate is preferentially sorbed on hydrous oxides at pH values ranging from 4.0 to 7.0, whereas arsenite is preferentially sorbed at pH values from 7.0 to 10.0 [144, 160]. Desorption of arsenate from Fe-oxide surfaces takes place at alkaline pH values [172, 173].

Although it is generally believed that arsenic solubility increases as pH increases, some experiments have shown that the combination of high pH and the

presence of sulphates and carbonates may provoke coprecipitation of arsenic with the oxyhydroxides or sulphates [174], or even precipitation of calcium arsenates. That may be the reason that certain soils reach the maximum retention of As(V) at pH around 10.5 [175]. In basic and well oxygenated soils, arsenic solubility may be limited by its precipitation as calcium or iron arsenates [176]. In soils with high pH, carbonates may play an important role as adsorbents [177].

Eh

The influence of the redox potential of the media over the arsenic speciation and mobility is evident, and it is closely linked to pH effects [178]. Masscheleyn at al. (1991) found a lower arsenic solubility and predominance of As(V) in soils at higher soil redox levels (500 to 200 mV or pe+pH~ 10-18). At alkaline soil pH, the reduction of As(V) to As(III) released important proportions of As(III) into the soil solution [178]. Under moderately reduced soil conditions (0-100 mV, pe+pH ~ 7-9), they stated that arsenic solubility was controlled by the dissolution of iron oxyhydroxides [178]. However, it is not clearly elucidated if the arsenic mobilization is the consequence of the reductive dissolution of the iron oxyhydroxides or rather because of reductive desorption [113, 178-180]. Upon reduction to -200 mV, the soluble arsenic content increased greatly as compared to 500 mV due to the reduction of ferric arsenate compounds and the higher solubility of arsenite minerals compared to those of arsenate [180]. Although the iron hydroxides use to be the main arsenic scavengers, they are not the only adsorbents of this metalloid. The surfaces of clays, organic matter, manganese oxides… also retain arsenic, and therefore, they are also influenced by the redox conditions changes in soils and sediments [181].

Due to the complexity of the soil as a system, thermodynamic modelling approaches are useful in order to make predictions about the possible redox transformations of arsenic minerals and the subsequent mobility processes [163]. In this sense, Sadiq (1997) designed a model with thermodynamic solubility isotherms of some minerals that is depicted in Fig. 1.18. It shows that, under redox conditions more oxidized than pe+pH~5, iron arsenate is more stable than all As(III) minerals in the soils whereas in the most reduced soils, As(III) sulphides are the most stable minerals. In anoxic soil systems, As(III) oxides are less stable than sulphides. Solubility of all As(III) sulphides sharply responds to redox changes and varies in a narrow range [163]. Nevertheless, the presence of non expected arsenic species can not agree with the redox predictions, due mainly to the slow kinetics of some reactions and the possible redox transformations performed by certain organisms [113].

Another important aspect to take into account is that the predominance of the reduced arsenic species increases with the depth increase, due to soils and sediments usually become more anoxic with depth [113].

In summary, it can be deduced that arsenic presents relative mobility over a wide range of redox conditions, higher than for the rest of heavy metalloids and oxyanion forming elements, which entails an important potential hazard for the environment [113].

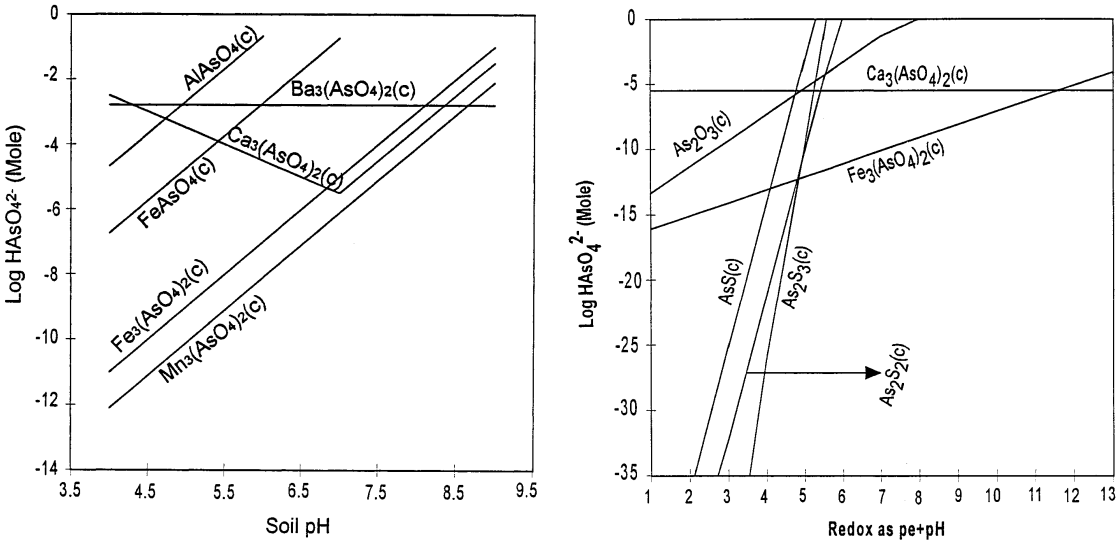

Fig. 1.18. Thermodynamic solubility isotherms of arsenic minerals in soils [163].

Fe, Al y Mn oxides/hydroxides, carbonates and arsenates

The presence of amorphous and crystalline oxides and hydroxides in soils and sediments provokes sorption or coprecipitation as the major mechanisms for arsenic trapping [182].

Regarding adsorption processes arsenate and arsenite are adsorbed onto surfaces of a variety of materials as metal oxides [173]. Iron oxides and hydroxides are the most important minerals involved in arsenic adsorption and desorption processes since they are widespread in the hydrogeologic environment as coatings on other solids [173, 183]. Al and Mn oxides also play a significant role when they are present in quantity [184, 185]. Its dependency on pH and Eh has been previously discussed. The kinetics of adsorption are dependent on both the concentration of the adsorbent and the adsorbing phase [114].

The kind of interaction that takes place between arsenic and oxides has been widely studied. Whereas some studies carried out by Extended X-Ray Absorption Fine Structure Spectroscopy (EXAFS) techniques on ferrihydrite [183] and goethite [186] reflects the existence of three different inner-sphere surface species of arsenic, either monodentate, there are also evidences about the existence of outer-sphere complexes [183, 187].

It has been stated that the presence of Al substituting Fe in the iron oxyhydroxides structures depresses the reductive dissolution of iron oxyhydroxides [188-190]. Arsenic adsorption capacity increases as substitution increases. Fe reduction in Al-goethites follows similar kinetics than non-substituted goethites. The fraction of Al substituting Fe may contribute not only to improve the arsenate adsorption capacity of soils and sediments, but also to diminish its mobilization under reducing conditions. Therefore, the Al-goethites present good potential as adsorbents to remove arsenic, from water [191]. Other authors have also found that the biological reduction of Al-, Cr-, Mn-, and Co-substituted goethites decreased as substitution increased [192].

As it seems that As(V) is adsorbed more strongly than As(III) onto the surfaces of minerals [193], a study about the degree of As(V) adsorption onto different oxides and hydroxides was carried out by Silva et al. (2010). They found that the mineral ability to hold arsenate increased in the following order: goethite (FeO(OH)), hematite (Fe_2O_3), gibbsite ($Al(OH)_3$), Al-goethite ((Fe,Al)OOH) and ferrihydrite ($(Fe)_2O_3 \cdot 0.5H_2O$) [191]. There are also studies that prove that As(III) may be adsorbed and oxidized by the surfaces of iron oxyhydroxides such as goethite, ferrihydrite, or birnesite ($(Na_{0.3}Ca_{0.1}K_{0.1})(Mn^{4+},Mn^{3+})_2O_4 \cdot 1.5\ H_2O$) [194].

In soils with very alkaline pH, carbonates can also play an important role as arsenate adsorbents [177]. Calcium carbonate minerals, especially calcite, can provide sites for arsenic retention [116, 132, 195]. Surface structural Ca^{2+} can interact specifically with arsenate, probably as outer sphere complexes [163, 196].

Arsenic often co-precipitates with iron oxyhydroxides due to its high affinity. Strong arsenic association with Fe_2O_3 is known to occur due to the stabilization and transformation of thermodynamically unstable amorphous iron oxide to either goethite or hematite [197]. Then, arsenic-bearing iron oxyhydroxides may act as an arsenic source in the case of reductive dissolution of both amorphous and crystalline oxyhydroxides.

Precipitation of other solid phases containing arsenic can also occur, especially in arsenic contaminated soils, where the weathering of primary arsenic minerals leads to the formation of other secondary forms, thermodynamically more stable. As a general rule, the major arsenic secondary minerals present in soils and sediments are arsenates [167, 168]. The natural precipitation of hoernesite ($Mg_3(AsO_4)_2.8H_2O$) [198], calcium arsenates and calcium-magnesium arsenates in less extent [199], aluminium arsenates and scorodite ($FeAsO_4.2H_2O$) [200] have been reported in mine wastes. However, sodium arsenate is not commonly found, due to its high solubility in water. Iron and aluminium arsenates are the dominant phases in acid soils. They are less soluble than calcium arsenates, which predominates in any alkaline and calcareous soils [163, 201]. These phases can also control the solubility of arsenic, aluminium and iron arsenates in acidic soils, and calcium arsenates in alkaline soils.

Clays

Clay minerals have also been reported as arsenic sorbents [175, 185, 202-204]. Although arsenic retention in clays is less effective than in the oxides [205], this phenomenon is not negligible. Since clays are positively charged in their surfaces, it seems that the arsenic adsorption occurs through cations bridges, as calcium [206].

Some studies have shown that montmorillonite can adsorb more As(V) and As(III) than kaolinite, due to the higher surface charge of montmorillonite [207]. Other studies have reported that halosite and chlorite have greater ability to adsorb As(V) than the rest of the clay minerals, and also that the kaolinite, illite and illite/montmorillonite present a moderate adsorption of As(V) [194]. Zhang and Selim (2008) suggested that isomorphic substitution of Fe by Al in clays can contribute to higher arsenic adsorption, as occurs with Al substitution in goethite [177].

Because of the adsorbent capacity of clays over arsenic species, the availability of this metalloid generally uses to be higher in sandy-textured soils than in clayey soils [111].

Organic matter

A great controversy exists around the role of organic matter in the retention of arsenic in soils and sediments. Some authors consider that contrary to what occurs with P, the association of arsenic with soil organic matter is not a predominant process in soils, especially when other soil constituents with higher adsorption capacity are

present in enough quantity, such as Fe oxides [33, 208, 209]. However, some other authors found evidences of rather high affinity of arsenic for soil organic matter [111, 210, 211] especially for humic acids (HA) and fulvic acids [111, 204, 210-214]. Even if it is assumed that humic substances sorb arsenic less strongly than hydrous iron oxides, they may, nevertheless, influence arsenic sorption and mobility, particularly when the iron oxide content in the environment is low [213]. Humic compounds are believed to be macromolecules spanning a wide range of molecular weights containing a large variety of functional groups, mainly phenolic groups, which may react with metals. Fig. 1.19 shows a detail of a humic acid. The compounds of smaller molecular weight and with larger proportion of functional groups will present higher solubility in water, whereas those more polymerized and with lower proportion of functional groups will remain stable over time in soils and sediments.

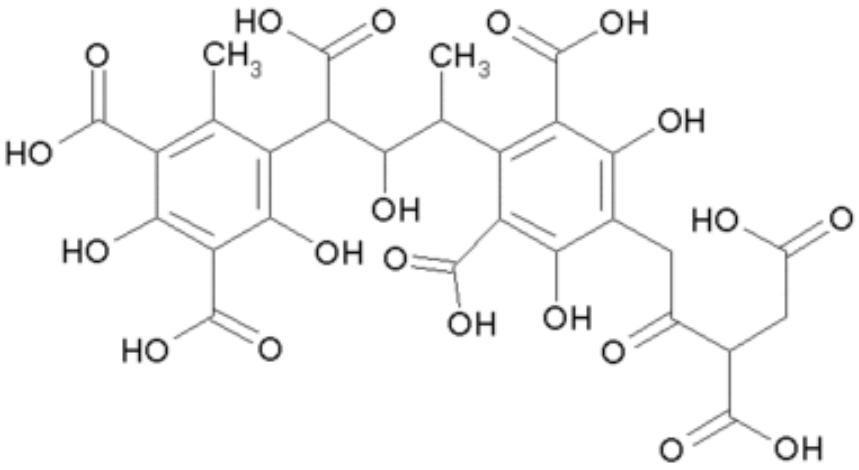

Fig. 1.19: Detail of the chemical structure of a humic acid.

Despite the predominant anionic nature of arsenic in soils and sediments, the interaction of As(V) with the carboxylic groups of the humic and fulvic acids is postulated to involve bridging cations such as Fe(III), Ca(II) with the deprotonated functional groups [215, 216]. Arsenite could form a negatively charged complex with a carboxylic group where H-bridges stabilize it. Regarding phenolic groups a ligand exchange mechanism might take place with arsenate [217]. These associations are dependent on pH, ionic strength and the concentrations of arsenic and humic substances [213]. It has been reported that the extent of the interaction is greater at the 8-10 pH range for As(V) while it is extended to pH 12 for As(III) [213]. The low extent of arsenic sorption at acid pHs (< 5), can be explained by the stronger protonation equilibrium of both arsenic species and humic acids [212]. For all arsenic species,

greater sorption occurred when their concentrations increased. Nonetheless, As(V) shows higher sorption capacity than the other arsenic species [213].

Organic matter may contribute to both arsenic mobilization or, on the contrary, to deplete its solubilisation. On one hand, the organic fraction with higher molecular weight that remain in the soil or sediment will retain arsenic, whereas the organic fraction that occurs as colloidal form may associate with arsenic provoking its solubilisation [218]. On the other hand, fulvic or humic acids form stable complexes with mineral surfaces since they are commonly found as anions [219, 220], effectively blocking arsenic adsorption. Therefore, competitive adsorption for binding sites on soil between arsenate and organic matter could inhibit the arsenic adsorption and propitiate its mobilization [140]. Likewise, simple aliphatic acids with low molecular weight excreted by plant roots (acetic, fumaric, oxalic, citric, etc.) might also mobilize adsorbed arsenic on oxyhydroxides by competing for reactive sites, or by dissolution of oxyhydroxides [221, 222].

Phosphate and other anions

The presence of adsorption-competing ions has also been shown to affect the arsenic mobility. Phosphate, in particular, has been well established as competitive with arsenic oxyanions in sorption onto iron oxyhydroxides [223-226]. However, the nature of the interaction between arsenic anions and phosphates for sorption sites, as well as the actual reactions or mechanisms between these chemicals at the surface of particles, is not completely understood. Several different models have been suggested related to layering of arsenic and competing ions on sorption sites on known minerals [171].

It seems that low phosphate concentrations do not represent an important impact on arsenic adsorption [171]. Reynolds et al. (1999) evaluated the effects of flooding on arsenic mobilization in phosphate amended soils. The authors found limited effects from the addition of phosphate on arsenic mobilization in their study of oxygen-depleted conditions, but indicated that higher concentrations of phosphate may have had a more significant impact [226]. However, they found through solid phase spectroscopy that the presence of phosphate decreased the fraction of arsenate in the solid phase. Phosphate may have had an indirect influence on the formation of sulphides and redox transformation of arsenate to arsenite. The results indicated that arsenopyrite formation was enhanced with phosphate amendment in the soils.

Arsenate desorption by phosphate may provoke abiotically reduction to arsenite, formation of sulphides, and the formation of arsenopyrite [171].

The practice of liming mine wastes to neutralize acidity and immobilize metals from the wastes and the practice of fertilizing locations with above background arsenic concentrations with phosphate fertilizers has also been studied [224, 225, 227]. The mobilization of arsenic observed with liming has been attributed to desorption effects from the increase of pH, resulting in competition between hydroxyl ions and arsenic. Changes in pH can also promote the release of arsenic following reductive dissolution of iron oxides where arsenate is known to be adsorbed. Jones et al. (1997) studied the effects on mobility of liming soils contaminated with arsenic (ranging from 48 to 3421 $mg \cdot kg^{-1}$) by a copper smelter. Liming increased the concentration of soluble arsenic in the effluent leached through columns filled with contaminated soils. The study showed that for the studied tailings, an increase of soil pH by liming provoked higher mobilization of arsenic by approximately one order of magnitude for a reprocessed tailing (pH increased from about 3.5 to about 8) and approximately two orders of magnitude for a pond tailing (pH increased from about 4.5 to about 9) [227].

In addition to phosphate, arsenic presents interactions with other anions. Thus, an increase in ionic strength of the soil solution can decrease the proportion of retained arsenic through competition between anions for the retention sites [205]. It is worth noting that the action of other anions appears to be significant only in the absence of phosphate, since when phosphate is present its effects will predominate. That is the case of carbonates, whose competition for retention sites with arsenic is negligible in presence of phosphate [228].

From all this can be deduced that the mobility and availability of an element in the environment is strongly influenced by its chemical form and the strength of the binding with the solid phases where this element is retained. It is evident that total concentration measurements do not reflect the stability of a pollutant. Sequential extraction procedures (SEP) provide a valuable tool for the assessment and identification of the element distribution among different solid phases, which it is known as fractionation according to the IUPAC [229]. They approach the extraction of the metal content into different operationally defined fractions by submitting the studied samples to successive reagents of increasing strengths. They pretend to simulate the conditions of environmental changes that could lead to the mobilization of the element,

such as acidification, oxidation or reduction processes [230]. Depending upon fractions of interest, a broad range of chemical extractants can be used and thus numerous sequential extraction schemes are available in the literature, which vary in the use of extractant, target phase and the order of addition to separate a particular form of metals. Since 1979 with the first sequential extraction scheme [231], numerous protocols have emerged, focus mostly in the partitioning of heavy metals [232]. The huge number of these procedures and the lack of uniformity among them makes impossible the comparison and integration of the results among different researchers [233]. Thus, in an effort to harmonize the diverse methodologies and to facilitate the easier comparison of results, the Bureau Community of Reference (BCR), now the Standards, Measurement and Testing Programme (SM&T) proposed a three step extraction procedure along with a reference sediment material to certify the protocol [234-239]. Although it was designed for heavy metals, it has also been occasionally applied to evaluate arsenic fractionation [142, 240-243].

Most of the SEP has been designed for the partitioning of heavy metals that mainly occur as cations. Conversely, arsenic is usually present in environmental solid samples as oxyanions, which implies that traditional SEP should be avoided for fractionation studies of this element and protocols especially designed according to its characteristics are needed [244]. For years an alternative to the mentioned traditional schemes have been the adaptation of soil-phosphorus fractionation procedures since arsenic characteristics resemble phosphorus behaviour. Hence, sequential extraction schemes specifically developed for P [245] have been adopted and conveniently modified for arsenic [246]. In the same way, SEP developed for selenium [247] have been conveniently validated for arsenic [248, 249] taking advantage of the chemical similarity between arsenic and selenium. Other schemes have been specifically developed for arsenic [168, 209, 250, 251], on the basis of the stability of its compounds over a smaller range of Eh and pH than that for P and its higher tendency to bound with S and C [252].

1.3.4.3 Remediation techniques for contaminated soils, sediments and spoil heaps

The selection of the most appropriate soil and/or sediment remediation method depends on the site characteristics, types of contaminants to be removed, concentrations of the pollutants and the end use of the contaminated medium [253].

Table 1.7. Summary of remediation techniques for contaminates soils and their associated costs [253].

Technology	Description	Applicability	Costs ($US/ton)
Containment			
Physical	Prevent movement by preventing fluid flow	Landfill covers and slurry walls	10–90
Encapsulation	Creation of an inert waste	Injection of solidifying chemicals	60–290
Vitrification	Application of electrical energy to vitrify contaminant	Shallow metal-contaminated soil, low volatility metals	400–870
Ex situ treatment			
Physical separation	Includes, froth flotation, gravity separation, screening, etc.	For high metal concentrations	60–245
Soil washing	Addition of surfactants and other additives to solubilize	For water soluble contaminants	25–300
Pyrometallurgical	Elevated temperature extraction and processing for metal removal	Highly-contaminated soils (5–20%)	200–1000
In situ			
Reactive barriers	Creation of a permeable barrier	Sorption or degradation of contaminants in barrier	60–245
Soil flushing	Water flushing to leach contaminants	For soluble contaminants	100–200
Electrokinetic	Application of electrical current	Applicable for saturated soils with low groundwater flow	Little info
Phytoremediation	Use of plants for metal extraction	Shallow soils and water	Good (50,000–200,000/acre)

The set of remediation techniques could be divided into three general categories: i) containment measures, ii) in-situ treatments and iii) ex-situ treatments. A summary of some remediation techniques is shown in Table 1.7 [253], although a wider range of remediation alternatives for soils and sediments has been described [254]. The first group refers to physical immobilization of contaminants in the original site. The two other remediation technologies implies to carry out certain treatments with the aim of reducing the level of contamination, and they can be performed in-situ, or ex-situ, which entails the transport of the contaminated land to other sites to be decontaminated.

Remediation technologies have their basis in physical, chemical and biological processes. Traditionally physicochemical treatments have been the most employed technologies, although they often provoke a high impact in soils and sediments. Hence, there is a current tendency of developing less aggressive methodologies, which in the case of arsenic is mainly focused on the use of adsorbents, predominantly iron oxides [255]. Similarly, biological techniques are being increasingly used due to the lesser impact provoked in soils and sediments; however, the dimension of the contaminated site and the longer time required by this kind of technology for remediation purposes makes it not appropriate for all cases. Biological techniques comprise two kinds of processes: i) those performed by microorganisms through reactions that convert toxic compounds present in the media in precursors for their cellular constituents, commonly known as bioremediation, and ii) phytoremediation or the use of plants to decontaminate sites. This last technology will be further discussed in section 1.3.6.4.

1.3.5 Arsenic in water

1.3.5.1 Arsenic abundance and legislation about water for human consumption and for other purposes

Arsenic contamination in waters is a crucial environmental problem, since drinking water is the main way of arsenic exposure to humans. Drinking water is derived from a variety of sources depending on local availability: surface water (rivers, lakes, reservoirs and ponds), groundwater (aquifers) and rain water. These sources are very variable in terms of arsenic risk [113].

Water contamination by arsenic is an important endemic problem in many parts of the world and there are numerous studies reporting extremely high arsenic concentrations in some natural waters [90, 92, 102, 113, 256-259]. In certain areas,

contaminated water is used for human consumption and for irrigation in agricultural zones, which has caused several health problems in the population of those sites as well as elevated arsenic concentrations in the foodstuffs that they consume [92, 259, 260].

Arsenic concentrations in fresh water range by more than four orders of magnitude depending on the source of arsenic and the local geochemical environment. Under natural conditions, the greatest range and the highest arsenic concentrations are found in groundwater. It tends to be found in two types of environment: firstly, inland or closed basins in arid or semi-arid areas, and secondly, strongly reducing aquifers often derived from alluvium. The greater tendency for arsenic accumulation in those ecosystems is consequence of the strong influence of water-rock interactions and the isolation of these water bodies. Arsenic-rich groundwater are also found in geothermal areas and, on a more localized scale, in areas of mining activity due to oxidation of sulphide minerals [113].

The range of arsenic concentrations differs widely among the different water bodies. Baseline concentrations of arsenic in rainfall and snow in rural areas are invariably low at typically less than 0.03 $\mu g \cdot l^{-1}$, although in areas affected by smelting operations, coal burning and volcanic emissions are generally higher. Arsenic concentrations up to 16 $\mu g \cdot l^{-1}$ have been reported near a copper smelter [131]. On a world scale, typical arsenic concentrations in non-polluted stream waters have been estimated in 4 $\mu g \cdot l^{-1}$ [261], but they vary according to the bedrock lithology. High concentrations of naturally-occurring arsenic have been found in river waters from geothermal areas and significant increases in river waters may also occur as a result of pollution from industrial or sewage effluents [261]. In the case of waters affected by mining activities these values can sharply increase. Arsenic concentrations up to 556 $\mu g \cdot l^{-1}$ were found in streams adjacent to tailings deposits in British Columbia [262]. Williams et al. (1996) and Smedley et al. (1996) reported around 200–300 $\mu g \cdot l^{-1}$ of arsenic in surface waters affected respectively by Sn in the Nakhon Si Thammarat Province [263] and because of Au mining in the area of Ghana [264]. Extreme arsenic concentrations have been reported in waters affected by acid mine drainage (AMD). Plumlee et al. (1999) found up to 340000 $\mu g \cdot l^{-1}$ in Various, USA [265]. Sarmiento et al. (2009) reported arsenic concentrations of 1975 $\mu g \cdot l^{-1}$ and 441 $\mu g \cdot l^{-1}$ for Tinto and Odiel rivers respectively in Spain [266]. Higher arsenic concentrations, up to 850000 $\mu g \cdot l^{-1}$ were found by Nordstrom and Alpers in surrounding waters of The Richmond Mine of the Iron Mountain copper deposit (California, USA) [267]. Concentrations of arsenic in

lake waters are typically close to or lower than those found in river waters. However, it is worthy to mention the extreme case of Mono Lake (California, USA), where up to of 10000–20000 $\mu g \cdot l^{-1}$ of arsenic were found, with pH values in the range 9.5–10 as a result of inputs from geothermal springs and the weathering of volcanic rocks followed by evaporation [268]. Average arsenic concentrations in open seawater usually show little variation and are typically around 1.5 $\mu g \cdot l^{-1}$. Concentrations in estuarine water are more variable as a result of varying river inputs and salinity or redox gradients. They are also usually low, with typical arsenic concentrations lower than 4 $\mu g \cdot l^{-1}$ under natural conditions. Background concentrations of arsenic in groundwater are less than 10 $\mu g \cdot l^{-1}$ in most countries. However, well-known high As-groundwater areas have been found in Argentina, Chile, Mexico, China and Hungary, and more recently in West Bengal (India), Bangladesh and Vietnam, exceeding the thousands of $\mu g \cdot l^{-1}$ of arsenic [269]. The scale of the problem in terms of population exposed to high arsenic concentrations is maximum in the Bengal Basin with more than 40 million people drinking water containing 'excessive' arsenic [113] and it has been estimated that around 100-200 million people worldwide are exposed to arsenic in drinking-water [116]. Fig. 1.20 exhibits the worldwide sites suffering important arsenic contamination in their waters.

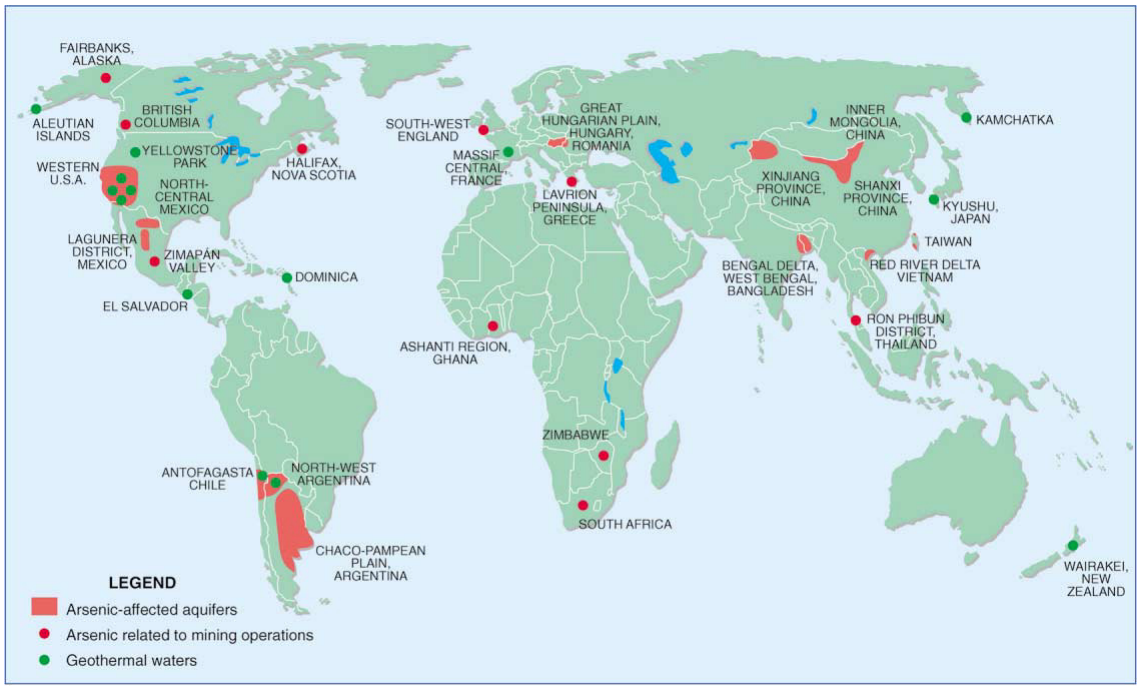

Fig. 1.20. Distribution of documented world problems with arsenic in groundwater in major aquifers as well as water and environmental problems related to mining and geothermal sources. Areas in blue are lakes [113] .

Until 2001, the long-standing legally acceptable limit imposed by the United States Environmental Protection Agency (EPA) for the concentration of arsenic in drinking water was 50 $\mu g \cdot l^{-1}$ (50 parts per billion, ppb). However, in 2001, the EPA revised this limit downwards, being established at 10 $\mu g \cdot l^{-1}$ (10 ppb). This decreasing followed the recommendations made by the World Health Organization (WHO) in 1993 [270], which were based on both an increased awareness of arsenic toxicity and the improvement of analytical techniques to quantitatively measure its concentration. However, in many parts of the world, including the most affected countries by arsenic contamination as India, Bangladesh and all other developing countries, this limit still remains at 50 $\mu g \cdot l^{-1}$. Nevertheless, the uncertainties surrounding the health impact of even very low arsenic concentrations have led the EPA to set a 'maximum contaminant level goal' at zero for drinking water. The European directive 98/83/CE also limited 10 $\mu g \cdot l^{-1}$ as maximum arsenic concentration in waters for human consumption [271]. In Spain, until 2003, the acceptable limit for arsenic in drinking water was also 50 $\mu g \cdot l^{-1}$, but the Spanish "Real Decreto 140-2003" announced 10 $\mu g \cdot l^{-1}$ as the maximum allowed arsenic concentration in drinking water [272]. For waters used for irrigation, a threshold of 100 $\mu g \cdot l^{-1}$ has been established following the recommendation of the FAO (Food and Agriculture Organization of the United Nations), whereas for mining and industrial effluents, the Spanish regulation limits total arsenic content to 500 $\mu g \cdot l^{-1}$.

1.3.5.2 Arsenic chemistry in water bodies

In natural waters, arsenic is mainly found in inorganic form as oxyanions of trivalent arsenite or pentavalent arsenate. Although the methylated compounds MMA and DMA may be produced as a result of microbial activity, they are rarely quantitatively important [113]. However, organic forms may occur where waters are significantly impacted by industrial pollution. Inorganic arsenic species dominate in sulphide oxidation environments [273].

The distribution among different inorganic species in water bodies is a function of pH and redox potential (Fig. 1.21). At moderate or high redox potentials, arsenic can be present as pentavalent oxyanions (arsenate): H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}. However, at more reducing conditions (acidic and mildly alkaline) and lower redox potential, predominate the trivalent arsenic species (H_3AsO_3) [110, 274]. The distribution of the species as a function of pH is given in Fig. 1.22. In addition to the importance of the oxidation state of the arsenic species, the degree of protonation can not be overlooked, since it will determine the interactions of arsenic with the dissolved substances and with the solid phases of the sediments in contact with the water body.

Arsenic mobility is directly related to its speciation. Therefore, it is also influenced by pH and redox potential. Contrary to heavy metals and to most oxyanions, arsenic tends to become less strongly sorbed as the pH increases [173]. The behaviour of arsenic also differs from the rest of metalloids and oxyanion forming elements in its relative mobility over a wide range of redox conditions, which makes arsenic the most hazardous among them. In the presence of extremely high concentrations of reduced S, dissolved As-sulphide species can be significant. Reducing, acidic conditions favour precipitation of orpiment (As_2S_3), realgar (AsS) or other sulphide minerals containing coprecipitated arsenic. Therefore high As-waters are not expected where there is a high concentration of free sulphide [14].

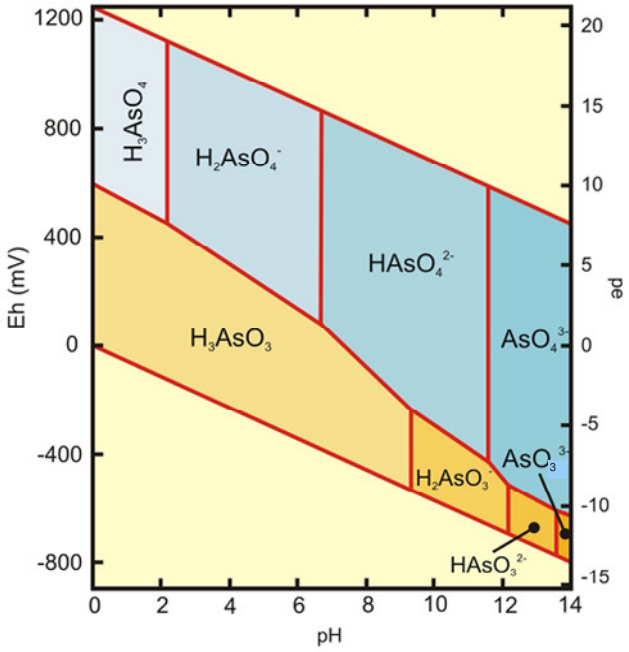

Fig. 1.21. Eh-pH diagram for aqueous arsenic species in the system As–O_2–H_2O at 25 ºC and 1bar total pressure [113].

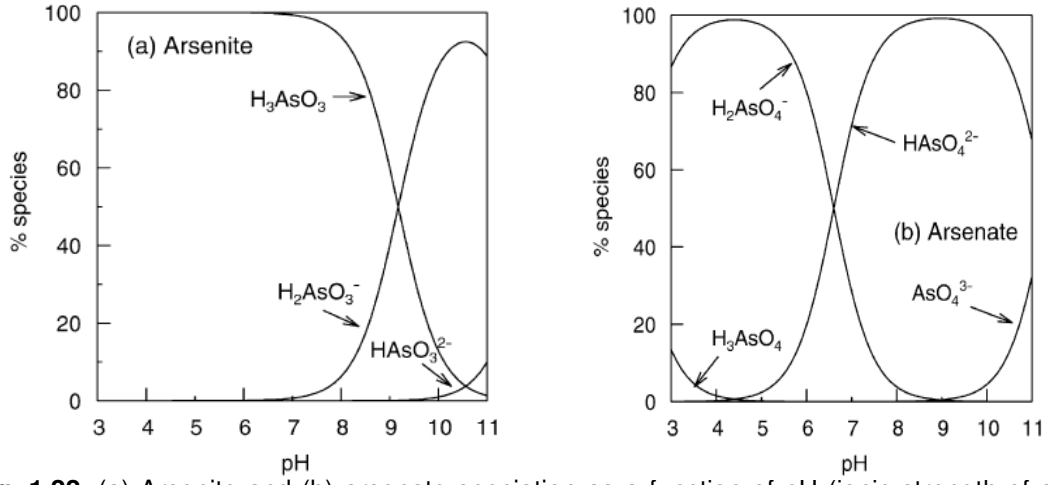

Fig. 1.22. (a) Arsenite and (b) arsenate speciation as a function of pH (ionic strength of about 0.01M) [113].

Arsenic speciation and solubility in waters affected by acid mine drainage (AMD) also exhibits dependency on pH and redox conditions of the site, as well on iron speciation. AMD is a special situation that occurs in mine sites with predominance of sulphides. During periods of excess oxygenation pyrite and As-bearing sulphides are dissolved allowing arsenic to enter the aquifers [263, 275-277]. The production of iron(III) and hydrogen ions can catalytically dissolve secondary minerals, thereby increasing the metal load to the drainage [265, 277]. In addition, due to the generally low pH of AMD systems (~1–4), metal dissolution can reach toxic levels. Roussel et al. (2000) and Daus et al. (2002) postulated that arsenic speciation is controlled by the pH-affected speciation and solubility of iron [278, 279]. For waters affected by AMD, Bednar et al. (2005) suggested that at pH greater than ~ 3.5 the Eh–pH relation shows that Fe(II) is strongly susceptible to oxidation to Fe(III), then precipitating as an oxyhydroxide or oxyhydroxysulphate. Since arsenite presents lower sorption degree onto oxyhydroxides than arsenate, arsenite species are dominant in solution in the higher pH drainages. At pH less than ~3.5 substantial amounts of dissolved Fe(III) exist and arsenite is expected to be oxidized to arsenate. Therefore the predominant species will be As(V) [280]. These relations found for AMD are not always applicable to other natural systems [280] since AMD is rather unique among natural systems because a dominant redox couple [iron (II)/(III)] exists, whereas in many groundwater, surface water, and soil systems this might not occur.

As the factors influencing arsenic mobilization from soils and sediments to water have been extensively discussed in section 1.3.4.2, we will not deal with this again.

1.3.5.3 Determination of arsenic speciation in water

The main interest in the study of arsenic speciation arises from the different toxicity of arsenic species. According to the IUPAC definition, speciation of an element is defined as its distribution among defined chemical species in a system, and speciation analysis refers to the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample [229].

A tricky and crucial stage in the speciation studies corresponds to the sample preservation during the storage in order to avoid alteration of the original chemical forms until the subsequent analytical determination. There exist numerous studies at this respect [281-289]. It has been reported that arsenic preservation is dependent on the sample matrix [290]. The distribution of As(III) and As(V) species depends greatly

on the abundance of redox-active solids, especially organic carbon, the activity of micro-organisms, and the extent of diffusion of O_2 from the atmosphere. Filtering the sample removes most of the colloidal material and micro-organisms that can affect the dissolved As(III/V) ratio [170, 291]. It is also known that photochemical reduction of Fe(III) can provoke the oxidation of As(III) to As(V) [292] when a water sample containing Fe(III) at pH> 2 is exposed to light. Then, storage in dark until analysis is recommended to maintain the As redox distribution [292, 293]. Acidification prevents oxidation and precipitation of Fe and Mn hydroxides that may coprecipitate or adsorb arsenic [294]. Chelation of Fe and other metals with ethylenediamine tetraacetic acid (EDTA) has been reported as a good alternative to inhibit the formation of precipitates [285, 289, 295].

For speciation analysis hyphenated techniques are required, since an efficient separation of species is needed followed by the quantification with a highly sensitive detection technique.

High-performance liquid chromatography (HPLC) is one of the best techniques in separating arsenic species in water matrices. The usual mode of liquid chromatography for the arsenic speciation is based on ions exchange. Since the most common arsenic species in water samples are found as anionic forms, (arsenate, arsenite as well as DMA and MMA), an anion exchanger column is necessary for their determination. Buffers (mainly phosphate buffers) are usually employed as mobile phases at different pH ranging from 4.5 to 8.5, and they can be used both in isocratic and in gradient modes [296]. For the separation of cationic species, such as arsenosugars, cationic exchanger columns could be used [297, 298]. For the determination of anionic and cationic species together, two columns of different nature could be placed in parallel [299]. Nevertheless, as arsenic is mainly found as anionic species in natural water samples, an anionic exchange column is enough to properly separate all the arsenic species present.

As regards the detection technique, selectivity, sensitivity and speed are required, since arsenic species contents can be found at trace levels. On one hand, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is one of the most commonly used techniques, since it provides a great sensibility, multi-elemental capability and large dynamic range. Furthermore, the coupling with HPLC results unproblematic, since the usual flow rates for HPLC are compatible with the uptake flow rate of an ICP source. Nowadays, it is the most usual technique for arsenic speciation analysis in most laboratories. The principal drawback of this technique is its high cost.

On the other hand, hydride generation (HG), followed by spectrometry, is also very habitual. This technique is based on the formation of volatile hydrides by means of chemical treatment of the sample with a reducing agent, typically sodium borohydride. The generated hydride is carried out by an inert gas to the detection system, decomposed in atoms and measured by atomic absorption spectrometry (AAS) or by atomic fluorescence spectrometry (AFS). The advantage of this technique is that HG process allows the separation of the arsenic species by volatilization from the rest of matrix components in the sample. Then, spectral and chemical interferences usually encountered in the detection systems are essentially eliminated. However, the weakness of the technique is that several organoarsenic compounds do not form volatile hydrides, so derivatization processes to convert them in hydride-forming species are necessary. Microwave-assisted oxidation [300] and UV photo-oxidation [301] with potassium persulphate and sodium hydroxide have been successfully proved for this task. Among the possible spectrometry techniques, AFS is the most attractive one, because of its low detection limit and lower costs of acquisition and operation [19].

1.3.6 Arsenic in plants

1.3.6.1 Arsenic abundance in plants

Terrestrial plants can uptake the arsenic present in the soil solution and accumulate it in their tissues. Marine plants uptake this metalloid from sea water. Terrestrial plants growing in uncontaminated soil usually exhibit low arsenic concentrations. Values ranging from 0.003 to 5 $mg \cdot kg^{-1}$ have been reported for them [126]. As this study is mainly focused on arsenic contamination in terrestrial plants. Table 1.8 shows typical arsenic concentrations found in some terrestrial plants species growing in uncontaminated sites, among them some edible plants [126]. However, plants growing in polluted areas can accumulate high or even extreme arsenic contents, depending on the location and pollution source, as it has been confirmed in plants growing in mining environments, in soils treated with arsenical pesticides and in soils with arsenic added by sewage sludge treatment [302]. For instance, values up to 6000 $mg \cdot kg^{-1}$ have been reported for arsenic concentration in plants from mining sites [303]. In areas where severe arsenic pollution occurs and contaminated water is used for irrigation, crop quality and yields have become a major worldwide concern [304]. Rice is usually the major crop in those affected areas. In many South-Asian countries it has been reported to accumulate up to 2 $mg \cdot kg^{-1}$ arsenic in grains [305] and up to 92 $mg \cdot kg^{-1}$ in straw [306] whereas total arsenic content in rice from non-polluted areas is normally ranging from 5 to 250 $ng \cdot g^{-1}$ [307]. The maximum concentration allowed for

total arsenic in foodstuff is 1 μg·g^{-1} [308]. The Food and Agriculture Organization/ World Health Organization (FAO/WHO) recommended a provisional tolerable weekly intake not higher than 15 μg of inorganic As per kilogram of body weight [309], which for an adult of 50 kg or a child of 20 kg (around 8 years old) would correspond to 107 and 43 μg·day^{-1} respectively [260]. It is well known that rice is the base of the diet in this area, and according to Roychwdhury et al. (2003) adults consume an average of 750 g of rice per day, whereas the daily average consumption of children is of 400 g [310]. Sanz et al. (2007) found an average concentration of inorganic arsenic in rice from arsenic-affected areas of Middle and Lower Ganga plain of 350 μg·kg^{-1} [260]. Although this value is below the maximum concentration allowed for foodstuff, it represents 263 μg·day^{-1} of arsenic intake for adults and 140 μg·day^{-1} for children, being those levels.5 and 3.3 times higher than the recommended values for adults and for children respectively [260]. Hence, the diet of people from those affected areas is of great concern [311, 312].

Table 1.8. Natural arsenic concentrations in some terrestrial plants. Modified from Matschullat (2000) [126].

Medium	Concentration (mg·kg^{-1})
Plants, terrestrial[a]	< 0.1–5 (0.65)
Mosses and lichens	0.26
Bryophytes	0.2–7
Mushrooms	1.2–2.5
Equisetum	0.2
Ferns	1.3
Grass	0.020–0.160
Wheat grain	0.010–0.070
Brown rice grains	0.110–0.200
Oat grains	0.010
Barley grains	0.003–0.018
Clover	0.280–0.330
Vegetables	0.01–1.5
Cabbage leaves	0.020–0.050
Kale	0.12
Carrots	0.040–0.080
Lettuce	0.020–0.250
Potatoes	0.030–0.200
Woody gymnosperms	0.2–1.2
Woody angiosperms	2
Spruce bark	10

The degree of arsenic accumulation also depends on the type of plant species in question. Leafy vegetables are the plant species believed to accumulate arsenic in greater extent, opposite to fruits, which are in the lower range [302]. Mushrooms are found to be relatively high arsenic accumulators [313]. McSheehy and Szpunar (2000) indicated that some sea plants are likely to concentrate a great proportion of arsenic from water [314].

The hyperaccumulators plants constitute a special case. They are plant species with the ability of assimilate great amounts of arsenic. In addition, conversely to what occurs for the rest of plants, whose roots are responsible for accumulating the bulk arsenic [315, 316], hyperaccumulators plants translocate the majority of arsenic from roots to fronds [317-319]. Some of the hyperaccumulator plant species described in the literature are *Pteris vittata* (up to 22000 $mg \cdot kg^{-1}$ total arsenic concentrations reported), *Jasione montana* (up to 6640 $mg \cdot kg^{-1}$), *Calluna vulgaris* (up to 4130 $mg \cdot kg^{-1}$), *Agrostis tenuis and stolonifera* (up to 10000 $mg \cdot kg^{-1}$), Pityrogramma *calomelanos*, *Mimosa púdica*, *Melastoma malabratrhicum* (up to 8350 $mg \cdot kg^{-1}$) [215] or *Dryopteris filix-mas* [320]. *Pteris vittata* and *Agrostis castellana* have been reported as two of the most common arsenic hyperaccumulator plants in Spain [321].

1.3.6.2 Arsenic speciation in plants

Arsenic is predominantly present as inorganic forms in terrestrial plants, although methylated arsenic species have been identified as well [39]. However, the so-called “arsenosugars” are the predominant arsenic species in sea plants.

Speciation analysis usually are carried out by hyphenated techniques coupling a separation technique and an on-line spectrometric detection system, as it has been detailed in the section 1.3.5.2 for waters. The same techniques would be useful for plant samples. For terrestrial plants, anionic exchange columns would be advisable for the separation of the corresponding arsenic species, since they are mainly anionic forms. For marine plants, whose predominant arsenic species are usually arsenosugars, cationic exchange columns would be preferred [322].

The determination of the arsenic speciation in plants needs the prior extraction of those species from the matrix in question. This can suppose the main problem of the whole process, since an appropriate extraction method that avoids contamination, losses and changes of the speciation is essential [323]. The diversity in the methods indicates that there is no agreement among authors on the choice of the best extraction

conditions [324]. A desirable extraction method should quantitatively extract all the arsenic species without altering their original speciation. The solvents and the employed conditions should not interfere with the species analysis [325]. Therefore, mild extraction conditions are required. For this purpose, different solutions have been used. Among them, mixtures of methanol-water (at different percentages of each one) [19, 326] and orthophosphoric acid (at different concentrations) [315] have been the most popular. Other combinations such as acetonitrile–water [327], methanol–water–chloroform [328] were also used. Usually those extractions were performed by physical shaking, sonication, pressurized liquid extraction or microwave-assisted extraction [329]. Other extraction procedures, such as Soxhlet extraction [330] and solid-phase extraction have also been described. Recently, enzyme digestion in combination with extraction was explored to improve extraction efficiency for some biological samples [331]. Extraction efficiency varies widely depending on matrix and extracting agent. Verification of the species stability can be assessed by adding the target species to the samples submitted to the considered extraction method [315, 332, 333].

1.3.6.3 Arsenic uptake, translocation and bioaccumulation in terrestrial plants

Available arsenic is the only soil arsenic fraction which can be uptaken by plants. It is obvious that the most retained arsenic in the solid phases can not be considered available to plants. There have been some attempts to determine the “bioavailable” arsenic fraction by means of extraction agents. The greatest difficulties lie on the simulation of the real uptake by plants. Tested approaches to evaluate the “bioavailable arsenic fraction” include both extractions in one step with certain saline solutions and suitable sequential extractions [157, 334-336]. Recently rhizoprobes are also being used to monitor the bioavalaible fraction by means of the determination of arsenic in the soil pore water [157].

The mechanisms of arsenic uptake and distribution along the plant are not completely established. However, in the last years the increasing interest in the biochemistry of arsenic in plants has led to numerous research studies.

The main route of arsenic entry into the plant is through roots, similarly to nutrients and other trace metals, although adsorption of airborne arsenic deposited on the leaves also occurs [125]. The arsenic compounds need to be solubilised in order to be absorbed by roots [337]. Carrier proteins are present in cell membrane to transport molecules and ions inside the cell but no specific carriers have been reported for arsenic yet. It is believed that the main entry of As(V) into the cell is through the

phosphate transporters as a phosphate analogue, whereas As(III) is transported in the neutral $As(OH)_3$ form through aquaglyceroporins carrier and through the phosphate transport system [338-341]. The largest proportion of arsenic taken up by plants is in the form of inorganic As(V), since in aerobic soils arsenate is the most stable and dominant species [319]. Some authors have reported that, although the absorption of organic arsenic compounds is less effective due to these species are much less common in soils, this phenomenon occurs [162, 342, 343]. However, other authors do not find evidences to elucidate whether the organic arsenic compounds found in plant tissues are simply taken up such as from soils or whether they were converted from inorganic species in soils to methylated forms in the plants [333]. Which seems clear is that under the reducing conditions of plant cells As(V) is mostly reduced to As(III) at the expense of reduced glutathione and catalyzed by arsenate reductase [44]. Then it is complexed by organic ligands such as thiols, and sequestered in the vacuoles of root and shoot tissues to avoid damage to plant cells [319, 344]. This process is depicted in Fig. 1.23.

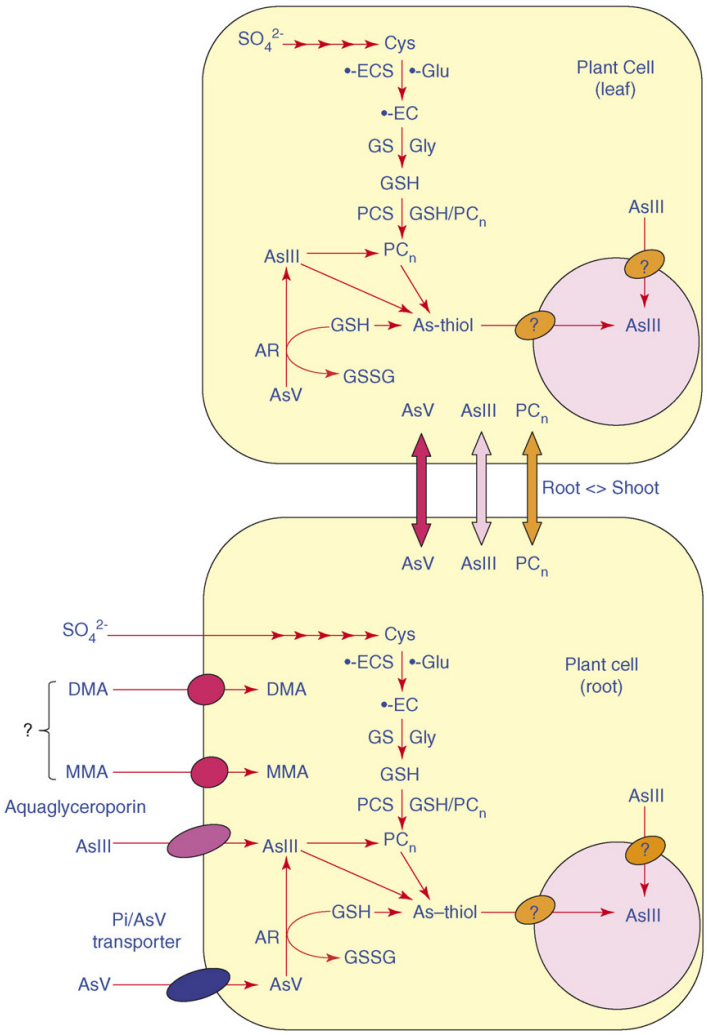

Fig. 1.23. Mechanisms of arsenic uptake, translocation and detoxification in plants [345].

There is discussion on the form in which arsenic is transported from root-to-shoot. Some studies performed with hyperaccumulators plants showed that arsenic is translocated to the shoot mainly as As(V) and is stored in the fronds as inorganic As(III) [346-348]. In contrast, Duan et al. (2005) found evidences that arsenate reductase activity is found exclusively in the roots, and hence, arsenic is translocated to the fronds in its reduced form [345, 349].

1.3.6.4 Phytoremediation of contaminated soils

The concept of phytoremediation of As-contaminated sites was proposed almost thirty years ago by Chaney (1983) [350]. It consists on the use of plants, soil amendments and agronomic practices to remove, retain, or reduce the presence of toxic contaminants on the soil [351]. In this section only the use of plants for remediation techniques will be tackled.

The strategy of phytoremediation covers different types of techniques [352-354], depicted in Fig. 1.24. Phytovolatilization, as the transpiration capacity of a plant species to volatilize some compounds, has been widely studied for other elements, such as mercury or selenium; however, this technique is not very consolidated yet in the case of arsenic [162]. The most important phytoremediation techniques applied to arsenic contamination episodes are phytoextraction and phytostabilization. The former one is based on the ability of certain plant species to absorb significant amounts of arsenic from the soil solution through roots and its translocation and accumulation in the aboveground biomass. Then, they will be harvested and properly managed [112]. This practice is called “induced phytoextraction” when certain crops of high biomass are induced to accumulate high arsenic concentrations by the application of some chemical amendments to their corresponding soils. The second variant of this practice, called “continuous phytoextraction” requires hyperaccumulator plants [317, 344, 355, 356]. The same plant specimen can be continuously employed if only the aerial part is cut down when it has reached the appropriate size and level of arsenic accumulation. Phytostabilization is defined as the immobilization of a contaminant in the soil through its absorption and accumulation in roots or rizosphere of certain plants, or through its complexation or precipitation in the rizosphere. The result is the reduction of the mobility and bioavailability of the pollutant. This term also refers to the use of plants for avoiding the migration, lixiviation or spread of a pollutant provoked by the erosion and weathering phenomena of wind and waters. Hence, the practice of revegetation and the presence of a vegetal cover prevents the spread of the contaminant by minimising erosion, runoff and percolation processes, thus reducing the

possible mobilization of the contaminant to groundwater [357]. Phytostabilization occurs due to the alteration of the microbiology and chemistry of the rizosphere and/or the soil, and the alteration of the pollutant. The soil pH may change because of the plant exudates and the production of CO_2, and consequently soils may transform soluble arsenic forms in other less mobile species [112]. Autosustainable plants with a long life and well adapted to the environment are required for the application of this remediation technology.

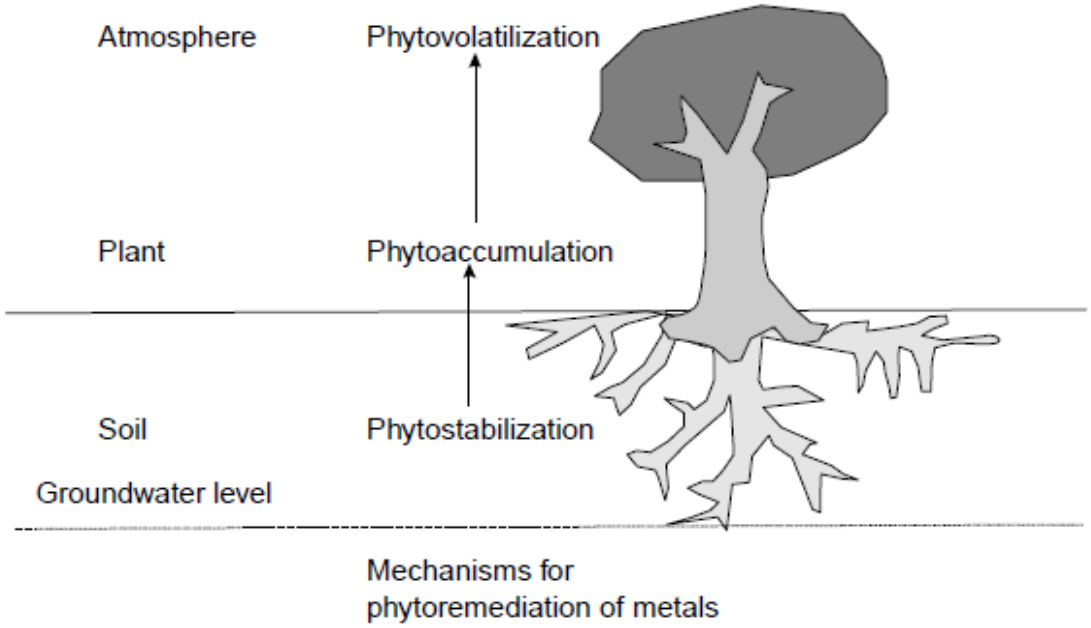

Fig. 1.24. Schematic diagram showing the mechanisms of the phytoremediation process for arsenic uptake [253].

Phytoremediation presents advantages over conventional remediation technologies of As-contaminated soils. The other currently available technologies are expensive, time consuming, can create risks to workers and produce secondary wastes [353, 358]. Thus, phytoremediation has the potential to become an environmental friendly and low-cost alternative remediation technique [304].

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2. STUDY SITE

Asturias is a region located in the North of the Iberian Peninsula where abundant mercury deposits exist. Although they are not as well known as Almadén deposits, Asturian mercury deposits have been historically exploited [1-11]. The extraction of mercury in this area dates back to Roman Times (ancient remains that allow to assign mining workings to that period have been found [11]). Fig. 2.1 shows the geographical distribution of the main Asturian mercury deposits.

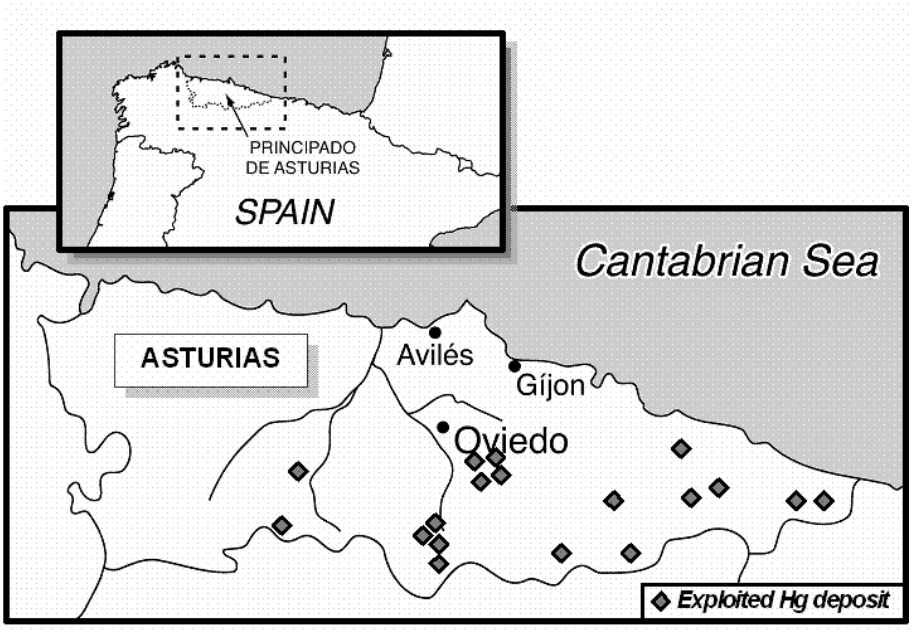

Fig. 2.1. Location of the main mercury deposits in Asturias [10].

In 1825, the Spanish "Real Decreto" about Mining Law provokes a resurgent interest in mining in Spain, with a big boost for mercury mining. In Asturias, the oldest mining works on record are located in the area of La Peña, along the valley of Miñera river, about 5 km North of the town of Mieres. In 1842, the first mining company in the modern era dedicated to the exploitation of mercury was located in Asturias. The purpose of this company was to extract and roast the recently discovered cinnabar deposits in La Peña valley. From this date, several companies were created to exploit the different mercury deposits discovered in Asturias.

As an example, the annual mercury production in Asturias during the last two decades in the nineteenth century is plotted in Fig. 2.2 [2]. These data show the great importance achieved by the mercury exploitation in Asturias. For example, the company "Sociedad Minera del Porvenir" produced about 20463 mercury flasks in the period between 1843 and 1893 (a mercury flask contains 34.473 kg of this metal) [1].

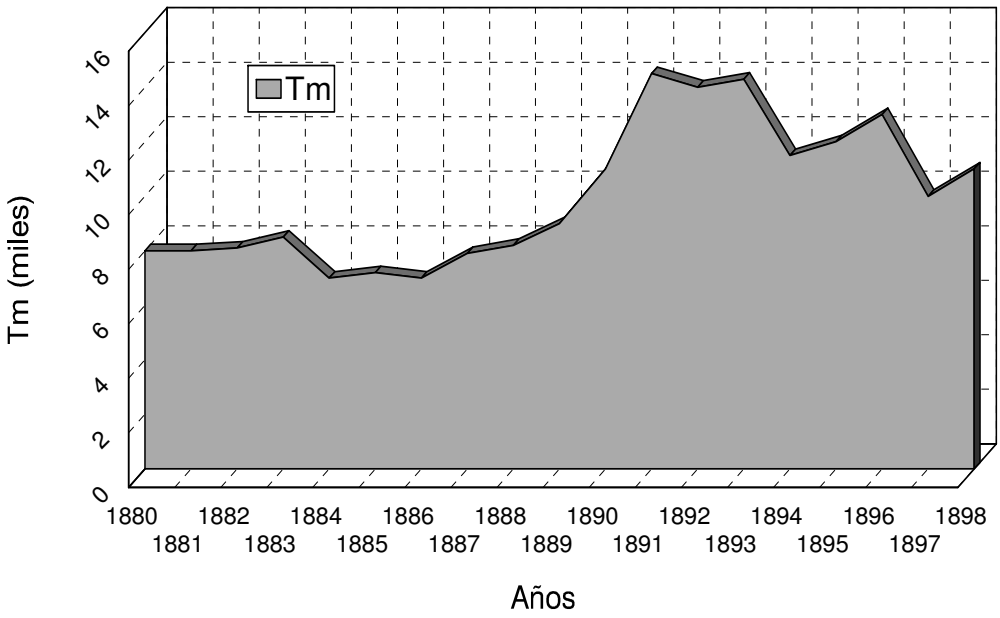

Fig. 2.2. Mercury production in Asturias during the last 2 decades of the nineteenth century.

In the early twentieth century, a significant crisis occurred at international level, which seriously affected the Asturian mercury mining companies. This led to the closure of a large number of them. From the 1940's, exploitation was restarted in the different deposits along the region. The decade between 1962 and 1972 represented the most flourishing period of mercury mining in Asturias. During this period, the most important Asturian mercury mines (La Peña, El Terronal, Muñón-Cimero, Brañalamosa, Maramuñiz, Piedracea, Olicio, Caunedo, etc.) achieved the peak of mercury production, reaching 5% of global production [3].

From 1972, the decline of mercury price in the world, due to international health concerns over this metal, gave rise not only to the suspension of some mining projects in the area, but also the closure of all the active mines between 1973 and 1974. Fig. 2.3 illustrates the peak and decreasing of production of mercury in Asturias between the middle of sixties and the middle of seventies.

There is evidence of numerous mining works dedicated to mercury extraction in Asturias, but the most important mines in terms of volume of extracted material and, in consequence, possible environmental impact, are La Peña-El Terronal and Los Rueldos, both in Mieres district, and La Soterraña, in Pola de Lena district [5]. All of them had metallurgical facilities for the roasting and concentration of the ore, which have greatly influenced to enhance their environmental impact.

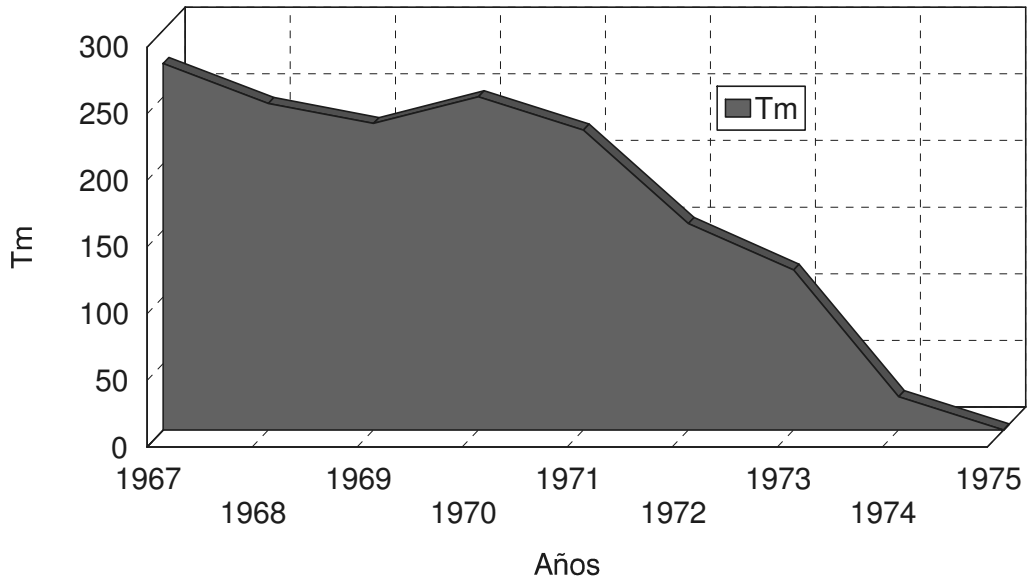

Fig. 2.3. Asturias mercury production (in tonnes) during the last decade of mining activity.

2.1 Mining areas in Mieres district

One of the studied areas is located in Northern Mieres, capital of the homonymous council, between the mine sites of La Peña and El Terronal, which is located 1.5 km from Mieres. The location of those mine sites, as well as the associated spoil heap (1, 2 & 4) are shown in the next figure.

Fig. 2.4. Mining areas in Mieres (1: La Peña; 2: El Terronal; 3: Los Rueldos; 4: Spoil heaps of La Peña-El Terronal group; 5: Nothern part of Mieres city; 6: Caudal river).

The access to the main shaft called Esperanza is carried out through a local road that links La Peña with Lada along San Tirso valley. The modern extraction of mercury at La Peña-El Terronal site was made by means of underground mining techniques, being the mineralization exploited in 12 levels. During the last years of exploitation, a production of more than 500 flasks/month was achieved, constituting at this time the second most productive mercury mine in Spain and the eighth in the world.

Fig. 2.5. El Terronal. Current view of the old mining and metallurgical facilities.

Another mine site in Mieres is Los Rueldos, located in upper Morgao valley, approximately in the border line between this valley and Miñera river valley. The height of the adjacent spoil heap is about 500 m.a.s.l. The steep slope favours the transport of fine suspended particles from mine wastes to close surface waters [4].

Fig. 2.6. Los Rueldos. View of the spoil heap.

2.2 Mining areas in Pola de Lena district

The main mine site in this district was La Soterraña, which is located in the Southwest slope of Campusas Mount, about 600 m Northeast of Muñón Cimero village and near the Northern boundary of the town of Lena (see next figure for details).

Fig. 2.7. Mining area in Pola de Lena (1: La Soterraña mining works and mercury condensate conductions; 2: La Soterraña spoil heap; 3: Muñón Cimero village; 4: Pola de Lena-Riosa road, AS-231, PK 4).

The mine workings were carried out mainly below the 650 m.a.s.l. elevation. A pithead at this height allowed the access to Ulpino shaft, which was communicated with deeper exploitations at 624 m, continuing through three inclined planes to 341 m with ramps, flat or intermediate wells. Mining and metallurgical facilities are located in a hillside of high slope, above the village of Muñón-Cimero. The exploitation of the ore was conducted by underground mining, by the chambers and pillars method. Spoil heaps containing tailings and low-grade ore are currently in the vicinity of the mining facilities, on the slope of a valley which favours the transport and dispersion of pollutants. Ovens for roasting the mercury ore were also found near the mine shaft, where there were also condensate conduits and chimneys to exhaust smokes [6]. Rich ores were treated in metallic muffle furnaces while poor ores were roasted in retort ovens for pulverized minerals and shaft furnaces. In 1957 a tilting-type rotary furnace with a treatment capacity of 25-40 t/day was installed.

Fig. 2.8. Partial view of La Soterraña spoil heap.

Fig. 2.9. La Soterraña. View of the old metallurgical facilities.

2.3 Geology and climate of the area

The mined ore deposits of Mieres and Lena districts are hosted in Carboniferous materials of Westphalian age, and some of the most important deposits are associated to conglomeratic horizons or siliceous breccias, and impregnating fractured lutitic sequences with thick calcareous horizons. Mercury deposits are generally associated to fractured zones, and the mineralization is usually irregularly distributed both in veinlets inside conglomeratic-brecciated bodies, which usually show a lenticular morphology, and scattered inside the conglomerate matrix. Mineralization also appears either as irregular massive lenticular stocks in carboniferous limestones, either as irregularly distributed veinlets in fracture planes, and occasionally constituting

disseminations in the enclosing limestones and sandstones [5]. The substrate of the mineralized areas is mainly constituted by alternation of limestones, sandstones, shales and some coal beds. A comprehensive study of the geological characteristics of each mineralization was presented by Luque [5].

La Soterraña mine exploited an epigenetic-type ore deposit, originated from low-temperature hydrothermal solutions [6]. The paragenesis of the ore deposit is mainly constituted by cinnabar, orpiment, realgar, pyrite (usually with high arsenic concentrations), arsenopyrite, marcasite, and pararealgar, in a gangue of quartz and calcite [7]. In mine wastes, iron sulphides (pyrite, marcasite and pyrrhotite) are quantitatively the most important metallic minerals phases, which decompose in the spoil heap, and breakdown of these minerals dominates the geochemistry of the spoil heap leachates. They are stable under reducing conditions, but they become unstable under oxidizing conditions. Those abundant sulphides are typically in an advanced state of oxidation, with amorphous iron oxyhydroxides coatings, being also abundant crystalline mineral phases (dominated by goethite). Some relicts of cinnabar, arsenopyrite and sphalerite have been also found. The presence of realgar is frequent as well. Arsenic content of As-rich pyrites ranges from 0.11 to 4.93% and high arsenic concentrations have also been found in iron oxides such as goethite (up to 2%) [7].

In La Peña-El Terronal and Los Rueldos mines, the presence of mercury is generally in the form of cinnabar, although metacinnabar and native mercury are often found [4]. Other present metallic minerals in the paragenesis of the ore deposits are pyrite, sphalerite, marcasite, chalcopyrite, galena, stibine, realgar and, exceptionally native gold [8]. Arsenic is usually present in the paragenesis of the ore deposits as orpiment and realgar, and more frequently associated with pyrite. The presence of arsenopyrite is scarce and limited only to some deposits [5].

From a hydrogeological point of view, the substrate of the mineralized area can be considered predominantly impermeable with the exception of some thick limestone and sandstone bars which can constitute small aquifers. Water that does not evaporate or flows superficially is infiltrated on these aquifers, as well as on the more permeable colluvial materials and weathered shales, giving rise to a number of small springs. In the studied area, springs are scarce and mostly associated to limestone bars, whereas those associated to sandstones have very poor flow.

In contrast to other regions of Spain, Asturias has a humid and temperate climate characterized by abundant rainfall during a great part of the year. The annual

average maximum and minimum temperatures of the last twenty years are 17 ºC and 8 ºC respectively, and the annual average relative humidity ranges from 74 to 84%. Average yearly rainfall in the same period was 966 mm and potential evapotranspiration calculated by Thorntwaite expression is 691 mm/year. In consequence, the average annual effective precipitation is 275 mm/year. The average one hundred-year rainfall is 97.8 mm/day [4].

Mine wastes are heterogeneous in size and nature and, in consequence, their hydraulic properties vary greatly; waste piles are usually quite permeable and constitute a porous media where wastes are intermittently wet by meteoric waters and seasonal runoff. Mine wastes are exposed to weathering processes and rain water can easily find its way through them, so that unstable minerals such as some sulphides are oxidized and therefore metals and metalloids are leached and put into solution, with the subsequent formation of secondary weathering minerals. Weathering kinetics of sulphide minerals is dependent on the exposition to the leaching agent. A waste pile, made up of lots of relatively small broken-up chunks, has an enormous amount of surface area of exposed sulphide mineral susceptible to react when gets in contact with water and/or air. The abundance and distribution of acid producing and acid buffering minerals vary extensively on the different parts of the spoil heap, depending on the type of materials stored along the time. Rainfall events provide ideal conditions for the dissolution of secondary weathering products and the transport in solution of leached metals and metalloids to the surface watercourses or to the saturated zone. At the mentioned abandoned mine sites, the circulation of surface waters in oxidizing conditions through the underground mining works and spoil heaps promotes in some cases the formation of acid mine drainage and spoil heap acidic leachates with its typical reddish Fe-oxides precipitates.

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3. FRAMEWORK AND OBJECTIVES

In Asturias (Northern Spain), the abandonment of some mercury mines after their closures without any preventive measure to avoid the spread of contaminants to the surroundings has provoked an important problem of arsenic pollution in those areas. The "GEOCIMA (Gestión de sistemas hídricos asociados al cierre de minas: Prevención de la contaminación y aprovechamiento del agua de mina como recurso)" project has allowed the study of the episodes of mercury and arsenic pollution in those areas, and high arsenic concentrations in soils and sediments of those sites, surrounding surface waters and even in autochthon plants growing in those areas have been found. This project (GCL2006-08192) was the result of the collaboration between the "Universidad de Oviedo" and the "Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT)" with the financial support by the Spanish Ministry of Science and Education from 2003 to 2009.

This thesis, in the framework of GEOCIMA project, was conceived to carry out a study of the environmental impact provoked by the spread of arsenic pollution in some abandoned mercury mines in Asturias. The general objective of this work is the assessment of the potential hazard that this element entails to those ecosystems. It must be achieved through the evaluation of the mobility and availability that arsenic presents in soils and sediments, surrounding waters and autochthon plants growing in those sites. Since studies in this respect do not exist for those areas, it is essential to know the forms of occurrence of arsenic in those media.

Analytical tools are necessary to tackle this kind of studies. Sometimes, well established methodologies have been adopted for our purposes, whereas often the existing methods were not appropriate for our necessities. Hence, new analytical methodologies have been developed and optimized to achieve the proposed goals.

As specific objectives of the current research could be mentioned:

- Development of a sequential extraction procedure (SEP) for arsenic fractionation in solid environmental samples for the evaluation of arsenic mobility. An extensive literature review will be performed to select the most important solid phases retaining arsenic, as well as the more suitable extracting reagents to target the desired pools. Optimization of the operational conditions will be carried out when necessary. The suitability of the selected reagents for extracting the target phases will be checked with natural and synthetic As-bearing minerals or As-spiked matrices. The applicability of this scheme to

diversity of samples will be tested through its application to polluted samples by different arsenic sources.

- Comparison of the proposed SEP with other established protocols in order to check the suitability of the novel procedure for studies or arsenic partitioning in environmental solid samples and with consolidating purposes.
- Application of the proposed SEP to sediments and spoil heaps materials coming from some different mining areas. Arsenic distributions will be intended to be understood in relation to the mineralogy of the deposits and the extent of mining operations in every case, and the potential mobility of arsenic in each mine site will be evaluated.

- Assessment of the factors controlling arsenic release in waters of the surrounding of those mine sites, based on physicochemical characterization and statistical studies of waters upstream and downstream of each mine.
- Determination of arsenic speciation in waters from three abandoned Hg mining areas for the assessment of the potential toxicity of the species occurring.
- Evaluation of different preservation procedures to assure no alteration of the original speciation and the sample stabilisation over time.
- Investigation of possible seasonal variations of arsenic contents and the parameters influencing its speciation and mobilization (pH and Eh) by monitoring the studied waters during one hydrological cycle.

- Establishment of an appropriate extraction method for arsenic from different tissues of terrestrial plants with very high arsenic contents preserving the original speciation.
- Study of arsenic accumulation and speciation in differentiated parts of several plant species grown in polluted mining sites.
- Assessment of the bioavailable arsenic fraction in soils for plants uptake and study of arsenic transference from soils to plants by means of transfer factors.
- Evaluation of the extent of arsenic accumulation by the plant as a function of the plant species and the degree of soil contamination.

4. ARSENIC IN SOLID SAMPLES FROM MINING ENVIRONMENTS

This chapter deals with the arsenic contamination in soils, sediments and waste materials from some mercury mining areas from Asturias. It is widely accepted that measurements of total concentrations does not reflect the real hazard of a pollutant to the environment. For risk evaluation purposes the mobility and availability of an element must be studied. In soils and sediments arsenic mobility is related to its associations with the solid phases present in those matrices and the strength of bindings. In this sense, sequential extraction procedures constitute a very useful tool to evaluate mobility, since they attempt the partitioning of an element into the most important fractions retaining it. Most of them have been conceived for the fractionation of heavy metals. However, arsenic presents a different behaviour since it is a metalloid and usually occurs in soils and sediments as oxyanionic forms. Hence, the application of sequential extraction procedures especially developed for this element is recommended.

The first section of this chapter refers to the development and optimization of a novel sequential extraction procedure designed for the study of arsenic fractionation in environmental solid samples. The main solid pools retaining arsenic will be considered in this protocol, focusing on the most labile fractions since they are the most potentially available ones. A careful selection of the extracting agents for every target phase will be performed through an extensive literature review. Likewise, their selectivity will be tested with experiments with natural and synthetic As-bearing minerals and As-spiked matrices. With the aim of proving the convenience of this protocol for variety of samples, it will be applied to real contaminated samples from different arsenic pollution sources.

The second section of this chapter pursues the consolidation of the proposed sequential extraction procedure through the comparison with other two well established and recognized schemes. One protocol is the harmonized BCR, conceived for the study of heavy metals partitioning, and the other is that developed by Manful as an adaptation from a phosphorus scheme for arsenic fractionation. The assessment of the efficiency and suitability of those sequential schemes and their corresponding extraction steps to partition arsenic into the most important solid pools will be carried out by their application to some heavily polluted sediment samples from the studied mining sites. A physicochemical characterization of samples is essential to support the results of arsenic fractionation and to establish the protocol that achieves the most appropriate arsenic distribution pattern.

The third and last section deals with the assessment of the arsenic pollution and mobility in soils, sediments and waste materials from the studied mining areas. Arsenic fractionation in samples from those environments will be achieved through the application of the developed sequential extraction procedure. For the understanding of arsenic partitioning in relation to the characteristics of the site, a mineralogical and physicochemical study of the samples will be implemented. The combination of these studies provides a valuable approach about the environmental fate of arsenic in the studied mining environments.

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4.1 Development and assessment of a sequential extraction procedure for arsenic partitioning and application to samples from different pollution sources

ABSTRACT

A novel sequential extraction procedure (SEP) has been developed and optimized for arsenic partitioning in the most relevant phases present in environmental solid samples, with special emphasis in the most labile fractions. The proposed seven-step SEP, considers the fractionation of the following arsenic pools: 1) readily soluble As, 2) strongly adsorbed As onto mineral surfaces, 3) As associated with Al oxyhydroxides, 4) As bound to organic matter, 5) As incorporated into amorphous Fe oxyhydroxides, 6) As associated with poorly crystalline Fe (hydr)oxides and 7) As coprecipitated with refractory minerals. An extensive literature review was carried out in order to select appropriate extractants. Operational parameters as solid-to-solution ratio and extraction time were optimized for certain steps. Selectivity of the selected extracting agents was assured through experiments with natural and synthetic matrices of As-bearing minerals and As-spiked materials. Usefulness of the proposed SEP was evaluated by its application to five As-contaminated samples from different origins. Recovery of total arsenic ranged from 85 to 103%. Results revealed that arsenic fractionation is largely in accordance to the pollution source of samples and with their geochemical composition. Hence, it can be concluded that the method is appropriate for a great variety of environmental solid samples.

Larios, R.; Fernández-Martínez, R.; Rucandio, I., Development and assessment of a sequential extraction procedure for arsenic partitioning and application to samples from different pollution sources. **2011**. *Submitted for publication.*

4.1.1 INTRODUCTION

Arsenic is present in soils and rocks throughout due to geology and mineralogy of land. Its distribution is rather uniformly, so a wide range of arsenic concentrations can be found in uncontaminated soils, with a mean of 5-10 $mg \cdot kg^{-1}$ [1, 2]. However, arsenic accumulation can rise greatly because of human activities such as use of pesticides, combustion of fossil fuels, mining and smelting of arsenic containing ores [3].

Arsenic usually forms oxyanions due to its great affinity for oxygen. Arsenite (As(III)) and arsenate (As(V)) are the most abundant forms in the environment [4]. Arsenites are 10 times more soluble and mobile than arsenates and hence, more toxic [5]. In oxygen-rich environments and well-drained soils, arsenate species dominate ($H_2AsO_4^-$ in acidic soils and $HAsO_4^{2-}$ in alkaline ones) whereas under reducing conditions, such as regularly flooded soils, arsenite is more stable [5, 6].

The mobility and availability of an element in the environment is strongly influenced by its associations with solid phases in soils and sediments. The strength of binding of an element to soil or sediment is totally dependent on the pool it is bound to. Trace elements usually occur in solid phases in some of these forms: (i) occupying exchangeable sites as diffuse ion or as outer-sphere complexes; (ii) specifically adsorbed as inner-sphere complexes; (iii) bound to organic matter; (iv) (co)precipitated as pure or mixed solids; and finally present in the structure of (v) secondary or (vi) primary minerals [5]. It is evident that total concentration does not reflect the stability of a pollutant. For risk assessment purposes, the evaluation of mobility and their related availability are of great importance since toxicity is directly related to such features.

Sequential extraction procedures (SEP) have attempted to partition the amount of toxic elements from the most labile forms to the most refractory ones. For that, the environmental solid sample is sequentially submitted to reagents of different and increasing strengths, intending to extract only the target phase. The reagents are selected on the basis of their ability to remove analytes from the desired phase. Extraction steps also correspond with important changes in environmental conditions that could affect metal binding, such as acidification, oxidation or reduction [7].

However, limitations of these schemes should be taken into account. On one hand, sequential extractions can only distinguish among operationally defined fractions, which implies that the extracted concentration of an element in each step depends on

factors such as the selected reagents and the order in which they are used, extraction time, solid/liquid ratio and sample preparation and preservation [5]. On the other hand, this methodology suffers from lack of selectivity, since the extraction of a unique phase is practically unachievable in practice [8], sometimes because of incomplete dissolution of the intended phase by overburden of the extracting agents, sometimes because of partial dissolution of undesired phases, or due to readsorption- and redistribution phenomena [5]. In spite of that, SEP provide useful and valuable information not achievable by other way for numerous environmental studies.

Most of the SEP has been designed for the partitioning of heavy metals that usually occur as cations. Conversely, arsenic is usually present in environmental solid samples as oxyanions, which implies that traditional SEP should be avoided for fractionation studies of this element and protocols especially designed according to its characteristics are recommended [9]. For years an alternative to the mentioned traditional schemes have been the adaptation of soil-phosphorus fractionation procedures because As and P chemistry in soils are rather similar. Therefore, the protocol developed for P by Chang and Jackson (1957) [10] have been often adopted or modified for arsenic [11], as the scheme proposed by Manful (1992) [12]. In the same way, SEP developed for selenium [13] have been conveniently validated for arsenic [14, 15] taking advantage of the chemical similarity between arsenic and selenium. Other schemes have been specifically developed for arsenic [11, 16-18], taking into account on one hand the stability of its compounds over a smaller range of Eh and pH than that for P and on the other hand its higher tendency to bound with S and C [19].

The goal of the current research is the development and optimization of a SEP for arsenic fractionation in order to assess arsenic mobility taking into account the main As-bearing phases present in solid environmental samples. Especially the most labile fractions were aimed to be separated and quantified. This SEP is based on a literature review of the extractions steps for targeting the desired phases, optimising operational conditions when necessary. Likewise, suitability tests of the selected reagents for extracting the target phase were carried out with natural and synthetic As-bearing minerals or As-spiked matrices. Finally, five arsenic contaminated samples with different source pollution were submitted to the application of the proposed method with the objective of testing the applicability of this scheme to a great variety of samples.

4.1.2 EXPERIMENTAL

4.1.2.1 Reagents, materials and apparatus

HNO_3 (65% w/v), HCl (37.5% w/v), HF (48% w/v) and $HClO_4$ (70% w/v) and all solid reagents were obtained from Merck (Darmstadt, Germany). Extracting solutions were prepared with Milli-Q water (Millipore, Bedford, MA). All glassware and bottles were cleaned by soaking in 0.5 M HNO_3 for 2 days and rinsed 3 times with deionised water before use. 50 ml polypropylene centrifuge tubes were used for the extraction experiments.

Total arsenic and major element determinations in the different extraction solutions, in the control sample, in the assuring matrices and in the total fractions were analyzed by using inductively coupled plasma atomic emission spectroscopy (ICP-AES), with a VARIAN 735-ES instrument in radial configuration. Previously, the solutions were acidified with nitric acid to pH 2-4 when it was necessary.

Total contents in solid samples were determined after microwave assisted acid digestion performed by an Ethos One MW Oven (Milestone).

pH and Eh of soils and sediments was measured with a Pt-Ag/AgCl electrode (CRISON) on sample/water slurries at 1:2.5 ratio (w/v).

Total organic carbon was determined as NPOC (Non Purgeable Organic Carbon) with an analyzer TOC (Shimadzu–VCHS) by means of oxidative combustion and determination with a non-dispersive infrared sensor (NDIR).

Other equipment employed in the assistance of the extractions was a vortex mixer (Labnet Inc.) with variable speed, a bench-top centrifuge (Eppendorf 5804), and end-by-end shaker (Bunsen ARR-8), a drying thermostatized oven (Proeti S.A.) with a maximum adjustable temperature of 200 ºC, automatic agate mortars (Fischer Scientific Co.) and an automatic homogenizer (Spex Mixer/Mill Cat. 8000).

4.2.2.2 Sequential extraction scheme

The selection of the extraction steps in this scheme was made according to the most important arsenic pools in solid environmental samples. A through literature review was performed for incorporating the more appropriate extracting agents proposed from other SEPs for targeting the desired phases. Optimizations of extraction

operational conditions were carried out when necessary. A sediment sample from a mining area whose total arsenic concentration was 2.15% was employed as control sample.

The final sequential extraction method is summarized in Table 4.1.1. 0.5 g of solid sample was accurately weighed into 50 ml polypropylene centrifuge tubes and sequentially treated for obtaining the desired target phase. After the addition of the corresponding extracting agents, the mixture was vortexed. All the extractions were aided by agitation in an end-over-end rotatory shaker at 35 rpm. After each extraction, samples were centrifuged at 5000 rpm for 15 min. Supernatants were extracted by using a Pasteur pipette and the residues were washed with 5ml of ultrapure water (excepting for the first stage), vortexed, recentrifuged and separated again. The supernatants were combined, filtered through 0.45 µm cellulose paper and diluted with water to a final volume of 50 ml. Finally, the residues from F6 fraction were transferred to PTFE vessels and digested with a mixture of $HCl/HNO_3/HF$ in a microwave oven.

Table 4.1.1. Proposed sequential extraction method.

Step	Target phase	Extractant
F1	Readily soluble As	H_20, 30 ml, 24 h
F2	Strongly adsorbed As onto mineral surfaces	0.5 M Na_2HPO_4 (pH= 8), 40 ml, 8 h
F3	As associated with Al oxyhydroxides	0.5 M NH_4F (pH= 8.2), 30 ml, 15 h
F4	As bound to organic matter	0.1 M $Na_4P_2O_7$, 10 ml, 16 h
F5	As incorporated into amorphous Fe oxyhydroxides	0.2 M ammonium oxalate/oxalic acid (pH= 3), dark, 20 ml, 2h + 2h
F6	As associated with poorly crystalline Fe (hydr)oxides	0.2 M sodium citrate + 0.6 M sodium bicarbonate + 0.4 M ascorbic acid (pH=8), 40 ml, 21 h (*)
FR	As coprecipitated with refractory minerals	acid digestion in MW owen: mixture of 2.5 ml HCl + 7.5 ml HNO_3 + 6 ml HF

Every extracting volume is referred to 0.5 g of solid sample.
(*) This step should be made twice only for samples with As content >1000 $mg \cdot kg^{-1}$.

4.1.2.3 Method assessment: synthesis of some matrices and arsenic spiked samples

In order to assess the suitability of every selected extracting agent for leaching the target phase, some natural As-bearing minerals and synthetic phases were subjected to the proposed sequential scheme. Natural minerals as hematite, arsenopyrite, realgar and orpiment were provided by the Escuela de Minas of the

University of Oviedo. Ferrihydrite, pure goethite and Al-substituted goethite were synthesized by the method of Schwertmann and Cornell (1991) [20]. Ferrihydrite was prepared by mixing 100 ml of 0.3 M $Fe(NO_3)_3$ solution with 66 ml 5 M KOH. Suspension was vigorously stirred and centrifuged. Then, it was washed twice with 50 ml of ultrapure water and dried at room temperature. Finally, it was grinded in an agate mortar. For the pure goethite 100 ml of 1 M $Fe(NO_3)_3$ solution were rapidly mixed and stirred with 180 ml of 5 M KOH solution until precipitation of red-brown ferrihydrite. Then, the suspension was immediately diluted to 2 l. with ultrapure water and hold in a closed polyethylene flask at 70 ºC for 60 h. The yellow-brown precipitate of goethite was washed and centrifuged twice with 50 ml of ultrapure water. Finally it was dried in an oven at 50 ºC and pulverised in an agate mortar. Al-substituted goethite was prepared by mixing 100ml of 1 M $Fe(NO_3)_3$ with 30 ml of 0.5 M $Al(NO_3)_3$ and 176 ml of 5 M KOH. After dilution to 2 l with ultrapure water, the suspension was placed in an oven at 70 ºC for 14 days. Once the crystallization had occurred the product was washed twice with 50 ml of ultrapure water, centrifuged, dried at 50 ºC and grinded in an agate mortar. The spiked minerals ferrihydrite, hematite, goethite and Al-substituted goethite were prepared by addition of 50 ml of 2 $mg \cdot l^{-1}$ of Na_3AsO_4 solution to the corresponding mineral, following by dilution to 500 ml with ultrapure water. pH was adjusted to approximately 4 and suspensions were stirred for 96 h. Spiked minerals were washed with ultrapure water, centrifuged, dried and pulverised in an agate mortar.

A sample with a high organic matter content was required for incubation with As(V) following the general patterns of spikes preparation from Schwertmann and Cornell (1991) [20]. For this purpose, commercial humus was used as solid matrix. It was also spiked with calcium and iron. 10 g of this matrix were mixed with 100 ml of 1000 $mg \cdot l^{-1}$ Fe(III) solution as $Fe(NO_3)_3$, 50 ml of 1000 $mg \cdot l^{-1}$ Ca solution as $Ca(NO_3)_2$ and 50 ml of 2 $mg \cdot l^{-1}$ As(V) solution. pH was buffered to 10 with 1M KOH and diluted with water to the mark in a 500 ml flask. The mixture was stirred for 48h at room temperature, settled for 12 h and evaporated at 50 ºC for 5 days. Then the sample was washed with 300 ml of bidistilled water, centrifuged, dried in an oven at 50 ºC for 2 days and grinding in an agate mortar [20].

The total arsenic content in every As-bearing mineral as well as in the spiked matrices was determined after acid digestion in MW oven by means of ICP-AES.

4.1.2.4 Origin and nature of experimental solid samples

Samples of different origin, nature and with diverse source of arsenic pollution were submitted to the proposed sequential extraction protocol. All extractions were performed in triplicate.

The source of arsenic pollution in four of the studied samples is mining. Sediment sample S2 was taken from “La Soterraña”, an abandoned cinnabar mine in Asturias (Northern Spain). It is located in an open-air channel just downstream of the spoil heap and it is receiving runoff waters and leachates from the mentioned spoil heap. S9 is a sediment sample taken inside the gallery of another abandoned mercury mine from Asturias, “Los Rueldos”. This point is affected by acid mine drainage and it is receiving effluents containing fine material from the mine gallery. R1 is a tailing sample collected in the upper spoil heap of “Santa Águeda” mine, formerly dedicated to the arsenic extraction and processing and located close to the vicinity of Riaño, in the Spanish province of León.

Sample NIC belongs to a mining area known as “Mina la India”, in the Department of León in Nicaragua. It is an unexploited mine of volcanic origin with huge amounts of iron-rich ores containing appreciable amounts of gold. In this area there are several wells contaminated with arsenic and a great indiscriminate use of pesticides has been carried out. The sample was taken from the side of the main road of the site.

CUB is a superficial sediment sample from Cienfuegos Bay, in Cuba. This area receives the input of heavy metals and organic residues due to the intense industrial activity in the surroundings.

4.1.3. RESULTS AND DISCUSSION

4.1.3.1. Selection of extraction steps

F1: Readily soluble As

The first step should extract the most labile arsenic. In the case of heavy metals, this corresponds to weakly-sorbed metal species or exchangeable cations retained on the soil surface by relatively weak electrostatic interactions. As they can be released by ion-exchange processes, traditional sequential extraction methods for heavy metal partitioning commonly employ an electrolytic solution such as salts of

strong acids and bases or salts of weak acids and bases at pH 7 to leach them.[21] The most used reagents have been $MgCl_2$ [8, 22], CH_3COONH_4 [23-26], NH_4Cl [27], or $CaCl_2$ [28, 29]. Although arsenic does not usually occur as cationic forms in soils and sediments, electrolytic solutions have been also very employed for the extraction of readily soluble arsenic, as $MgCl_2$ [17], NH_4Cl [5, 30], based on Manful scheme [12], or KCl [14]. However, since arsenic prevails in soils and sediments in anionic forms, it seems more reasonable other extraction way for this first step. With this purpose an anion exchange membrane was tested [5, 31], but no good results were obtained. On the contrary, arsenate and arsenite pose high water solubility [32] and, in consequence, can be leached by water at neutral pH. Some authors found greater extracting capacity in water than in electrolytic solutions, as well as they proved the solubility in water of certain minerals containing arsenic, such as sodium arsenate [5]. Therefore, ultrapure water was chosen for the first extraction. Moreover, water-extractable arsenic can be considered bioavailable for plants, since some studies found notably correlations with water-soluble fraction and the uptake of arsenic by plants [33-35].

F2: Strongly adsorbed As onto mineral surfaces

It has been studied that the major minerals binding arsenic (as both arsenate and arsenite) in soils and sediments are the metal oxides, particularly those of Fe, Al and Mn [36, 37]. Although larger adsorption occurs onto Fe oxides or oxyhydroxides surfaces [2], Al oxides also play a significant role when it is present in quantity [38, 39]. It seems that the adsorption of As(V) onto mineral surfaces generally occurs in larger extent than the adsorption of As(III), and on the other hand, that in well-oxygenated soils arsenic is found mostly as arsenate [5, 6].

The kind of interaction that takes place between arsenic and oxides has been also widely studied. Whereas some studies carried out with EXAFS techniques on ferrihydrite [40] and goethite [41] reflects the existence of three different inner-sphere surface species of arsenic, either monodentate, there are also evidences about the existence of outer-sphere complexes [40, 42].

The reagent commonly used for the extraction of specifically-sorbed arsenic is phosphate. Kim et al. (2003) [14] and Keon et al. (2001) [17] used it as NaH_2PO_4, whereas Wenzel et al. (2001) [16] did it as $NH_4\ H_2PO_4$ and Cai et al. (2003) [43] as KH_2PO_4. The reason for this reagent is the great chemical similarity between arsenate and phosphate, but due to the smaller size and higher charge density of phosphate, it

outcompetes arsenate for adsorption sites in soils [44]. The selectivity of this reagent has been proved by the fact that only small amounts of Al, Fe and Mn were extracted in this step, indicating no significant dissolution of these oxides/hydroxides and its selectivity for surface-bound arsenic [16, 43]. The amount of arsenic that plants uptake has been also well correlated with phosphate-extractable arsenic from soils, indicating that this fraction can be considered also as bioavailable [35, 45].

F3: As associated with Al oxyhydroxides

The extraction of this fraction has generated controversy between different authors and methods. NH_4F has been always the chosen reagent for this purpose. The mechanism of this extraction is based on anionic exchange and the formation of highly stables Al-F complexes [46] and NH_4^+ and arsenate associations. It is not entirely elucidated if this reagent leaches arsenic adsorbed onto Fe and Al mineral surfaces, or arsenic coprecipitated with them, since precipitation-dissolution and adsorption-desorption reactions are not totally unrelated processes [2].

However, it has been criticized for being insufficiently selective or specific for the target phases or for its low extraction efficiency. For instance, Wenzel et al. (2001) [16] did not found microscopic evidences for As–Al association and they stated that arsenic extraction by NH_4F is not directly linked with the concurrent extraction of Al. Nevertheless, the NH_4F step that they essayed lasted for only 1 h. Maybe it is a matter of insufficient extraction time, since Van Herreweghe et al. (2003) [5] applied NH_4F extraction based on Manful method [12] with a duration of 15 h and they leached noticeable amounts of arsenic (> 25% of total arsenic in some samples).

Anyway, we have considered advisable and useful taking into account this fraction, since hydroxy-Al on the external surfaces of micaceous minerals has been reported to be especially significant in arsenic retention [47]. The order of this fraction in the sequential extraction scheme is in accordance to arsenic mobility, since it is known that Al-arsenates are more soluble than Fe-arsenates but less labile than phosphate extractable arsenates.

F4: As bound to organic matter

This step is also very controversial, since some authors consider that contrary to what occurs with P, the association of arsenic with soil organic matter is not a predominant process in soils, above all when existing other soil constituents in enough

quantity with higher adsorption capacity, such as Fe oxides [5, 16, 48]. However, some other authors considered that arsenic poses high rather affinity for soil organic matte r [49, 50] and Thanabalasingam and Pickering (1986) [51] found evidences of arsenic sorption onto humic acids in batch experiments. The association is dependent on pH, ionic strength and arsenic concentration. It has been reported that the extent of the interaction is greater in the pH range 8-10 for As(V) while it is extended to pH 12 for As(III) [52]. In principle, this fraction is supposed to be negligible in the surface horizons of soils, but it can be important or even dominate the arsenic partitioning in polluted soils and sediments, sewage sludge [53] or samples with high organic matter contents, such as forest soils [54].

Moreover, significant arsenic concentrations bound to organic matter were found when this step was applied to some soils [55] or mine wastes containing appreciable amounts of organic matter [14]. Also significant arsenic concentrations were found in the oxidizable fraction of BCR protocol when it was applied to the reference sediment BCR 701, sediments of different origins (lake, river and canals [56] and sediments near spoil heaps [57].

Many leaching solutions have been described in literature to extract metals associated to organic matter. Some of them pretend the degradation of the organic matter under oxidising conditions with the subsequent release of soluble trace metals [58, 59]. Among them, the most commonly used are H_2O_2 at acid pH [8, 60-62] and NaOCl at pH 9.5 [62-65]. The problem is the lack of specificity of both, since as well as the desired phase, other phases such as Fe and Mn oxides and sulphides may also be solubilised [66, 67]. Although the use of NaOCl minimizes the dissolution of amorphous constituents and clay minerals, it has been proved that in the case of calcareous soils partial destruction of carbonates occurs [68]. NaOH has been also employed in traditional sequential schemes for dissolving organic matter [28, 69]. Although it also can alter aluminosilicates and clays [61, 70] and this reagent leads to hydroxide precipitation, it is particularly used for fractionation studies in sludges, which essentially have a very high content of organic matter [28, 70]. However, when this reagent is employed for arsenic extraction, it does not extract arsenic bound to organic matter, but also arsenic associated to iron oxyhydroxides [15, 71, 72].

The use of $Na_4P_2O_7$ at pH 10-13 has been recommended by some authors because of its high selectivity to dissolve humic and fulvic acids [73-75]. It has been established that polyvalent cations (e.g., Ca^{2+}, Al^{3+}, and Fe^{3+}) are responsible for maintaining organic matter in a flocculated and insoluble state in many mineral soils

[54]. Pyrophosphate ions acts by forming metal complexes and promoting the dispersion of organic colloids and therefore, the solubilisation of soil organic matter [54, 70].

Hence, this reagent was selected for leaching arsenic bound to organic matter, as it seems the most selective among all. Some authors have suggested to carry out this step before the extraction of the moderately reducible fraction, mainly when the latter fraction is extracted with oxalate [76].

F5: As incorporated into amorphous Fe oxyhydroxides

Iron and manganese oxides are excellent scavengers of metals, being the amorphous minerals more labile than the crystalline phases [77, 78]. As it was pretended their differentiation by order of mobility, they may be leached sequentially. This can be achieved by controlling the Eh and the pH of reagents [79, 80]. The most successful reagents in the extraction of metals associated with these minerals contain both a reducing reagent and a ligand able to retain released ions in a soluble form [79]. Hydroxylamine and oxalic acid are the reagents more commonly employed for the leaching of metals incorporated into amorphous Fe oxyhydroxides. As regards hydroxylamine, extraction conditions must be carefully controlled to minimise the partial dissolution of other pools. Although Mn oxides are readily extracted, an increase of reagent concentration, extraction time or a pH too low could lead to dissolution of crystalline iron oxyhydroxides [81], or even cause partly the release of metals associated with the silicate fraction [82]. Then, a warm hydroxylamine solution is generally used at pH 2 for an extraction time ranging between 30 min and 6 h. Its preparation in acetic acid (25% v/v) or 0.25 $mol \cdot l^{-1}$ hydrochloric acid instead of in nitric acid has the advantages of the complexing properties of chloride or acetate ions [83]. The classical sequential procedures have often used this reagent for the extraction of this pool [24, 84]. It has been also employed in schemes adapted to arsenic [14]. Another reagent fairly selective for dissolving amorphous Fe oxyhydroxides is the oxalic acid/ammonium oxalate buffer (NH_4Ox/HOx) at pH 3, also called Tamm´s reagent. The extraction must be conducted in dark, since UV catalyzes the attack of crystallised iron oxides [20]. It has been widely used in sequential schemes for leaching the moderately reducible fraction for both metals [22, 23, 29, 76, 85] and arsenic [11, 17, 18, 86] being advantageous over hydroxylamine hydrochloride due to its higher complexing capacity [87]. However Tamm's reagent has been criticized by the possible formation of very stable complexes with aluminium [85], and by the leaching of metals

associated with organic matter [88]. Since the affected pools are previously leached in the proposed scheme, oxalate buffer was selected as extracting agent.

F6: As associated with poorly crystalline Fe hydr(oxides)

Sodium dithionite is a sufficiently strong reducing agent to dissolve even well-crystallised Fe oxides in the pH range 7–8 [89]. It is commonly employed together with a complexing ligand (usually ammonium citrate), and bicarbonate, to adjust the pH of the mixture. Several sequential extraction schemes have used it [29, 90]. Nevertheless, it presents some drawbacks. On one hand, commercial dithionite salts can be contaminated with zinc and a complex purification step is often necessary [8, 89], and on the other hand, substantial precipitation of sulphides or sulphates can occur.[85] The pool of arsenic incorporated in crystalline iron oxides has been also extracted with this reagent in some protocols, Manful (1992) [12] and Van Herreweghe et al. (2003) [5] in a modified Manful scheme. The problems of dithionite are solved by some authors with the addition of successive small portions of the solid, but it does not result practical from an operative point of view and it would impede the automation of the method.

As it has been mentioned above, this fraction can be also leached with Tamm`s reactive in presence of light [91]. Shuman (1982) [85] proposed the combination of Tamm's reagent, temperature and the effect of a supplementary reducing compound (ascorbic acid). In these conditions, the attack would be similar to that obtained with dithionite. It has been also employed in arsenic fractionation schemes [18]. Other authors preferred only the mixture ammonium oxalate/ascorbic acid [26, 92, 93]. Its advantage over the use of dithionite is the higher purity degree and that it does not attack silicates [85]. Other employed reagent has been 0.05 M Ti(III)-citrate-EDTA-bicarbonate at pH= 7 [17]. Its preparation implies the handling of Ti(III) chloride under air free conditions to prevent reactions with oxygen because of its riskiness, which does not result practical.

However, highly crystalline oxides can be considered refractory minerals because of its high stability. As this SEP pretends to establish a large differentiation among the labile phases, it was preferred to extract poorly crystalline hydr(oxides) and to relegate the refractory crystalline forms to the residual fraction. Hence, the mixture proposed by Paul et al. (2009) [94]. 0.2M sodium citrate/0.6M sodium bicarbonate/0.4M ascorbic acid (pH= 8.0), was considered suitable for the extraction of arsenic associated with poorly crystalline Fe hydr(oxides). Moreover, the replacement of

dithionite by ascorbate avoids the dithionite problems, and the use of this mixture avoids the extraction of labile sulphides and consequently it is more selective [94].

4.1.3.2. Optimization of extraction conditions

All the optimization experiences were conducted by using 0.5 g of control sample. The optimization of the extraction conditions are summarized in Fig. 4.1.1.

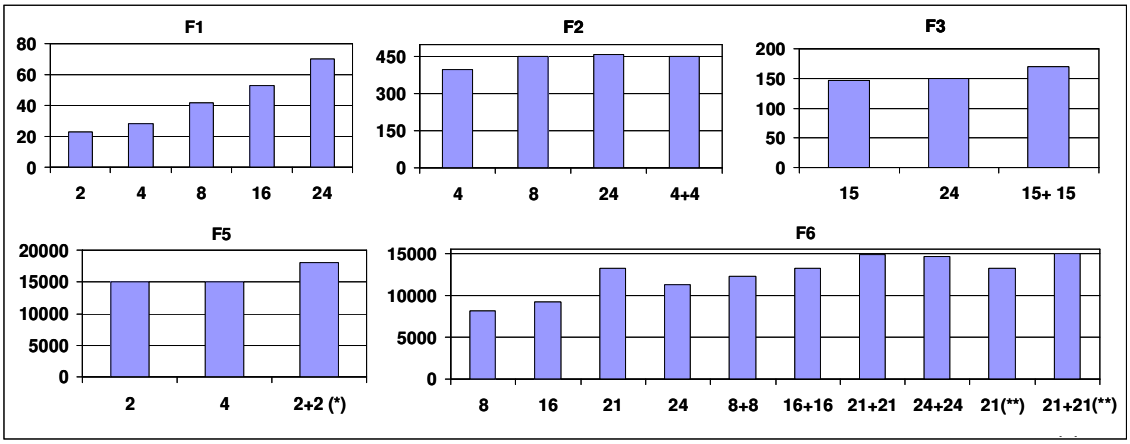

Fig 4.1.1. Optimization of extraction conditions in F1, F2, F3, F5 and F6 steps.

The arsenic extracted ($mg \cdot kg^{-1}$) is represented in y-axis, and the duration of each extraction (h) in x-axis. When two consecutive extractions are shown, it is expressed as 4+4, for example.
(*) These consecutive extractions were carried out with 20 ml of extracting agent.
(**) The extraction was performed with 60 ml of extracting agent.

4.1.3.2.1 *Readily soluble arsenic (F1): H_20*

The volume of ultrapure water was 30 ml, as Van Herreweghe et al. (2003) [5] proposed in their Scheme II modified from Manful (1992) [12]. Extraction times of 2, 4, 8, 16 and 24 h were tested. Extracted arsenic concentration increased with time, which means that water extraction is highly dependent on time. From an operational point of view, a longer extraction times is not practical. Hence, extraction time of 24 h was selected.

4.1.3.2.2 *Strongly adsorbed As onto mineral surfaces (F2): Phosphate*

Phosphate concentrations usually employed range from 0.05 M [16] to 1 M [17]: Since excess addition of phosphate is needed to replace arsenic anions, and some tests carried out with phosphate at different concentrations (from 0.05 M to 0.5 M) showed greater extraction with the highest concentration [16], 0.5M NaH_2PO_4 was assumed as reasonable.

According to Keon et al. (2001) [17], 40 ml of solution was considered convenient to ensure that the extractant did not become exhausted.

Initially, extraction times of 4, 8 and 24 h were essayed for 0.5 M NaH_2PO_4 (pH=8).

It seemed that the optimal extraction was achieved in 8 h. Moreover, to check if re-adsorption phenomena occurred, consecutive extractions of 4 were achieved. The leached arsenic concentration was similar to this obtained for 8 h. Hence, 8 h was the selected time.

4.1.3.2.3 *As associated with Al oxyhydroxides (F3): NH_4F*

As starting conditions, a solution of 0.5 M NH_4F at pH= 8.2 was selected, according to Manful (1992) [12]. Different extraction times were tested: 15 h, 24 h and two consecutive 15 h extractions. Whereas the obtained arsenic concentrations for 15 and 24 h were similar, the arsenic extracted in two consecutive steps of 15 h was slightly higher. However, the additional amount of leached arsenic by the second extraction, representing only 0.1% of total arsenic content of the sample, was not worthy for the time-consuming of the stage. Therefore, an extraction time of 15 h was considered suitable for this stage.

4.1.3.2.4 *As bound to organic matter (F4): $Na_4P_2O_7$*

The commonly used pyrophosphate concentration is 0.1 M [54, 75], Previous studies have demonstrated that 10 ml is an adequate volume of this extracting agent for 0.5 g of sample [95] and that the optimal extraction time is between 16 and 20 h. Then, according to Fernández-Martínez (2006) [95], samples were subjected to 16 h of extraction with 0.1 M $Na_4P_2O_7$.

4.1.3.2.5 *As incorporated into amorphous Fe oxyhydroxides (F5): ammonium oxalate/oxalic acid*

The extraction of this phase has been conventionally conducted with 0.2 M ammonium oxalate/0.2 M oxalic acid (pH=3) in dark [11, 17, 96]. However, solid-to-solution ratio and time extractions have been very diverse, depending on the schemes. For instance, Keon et al. (2001) [17] employed 40 ml of extracting reagent for 0.4 g of sample for 2 h, whereas Matera el al. (2003) [18] choose a v/s ratio of 50 and a duration of the step of 4 h. In view of the necessity of optimizing this step, the control

sample was initially submitted to a 2 h extraction with 40 ml of Tamm's reagent, based on the conditions employed by Keon et al. (2001) [17]. Moreover, another single extraction of 4 h was essayed, and no significant difference in the arsenic leaching existed among the two extractions. To assure that this was not due to oxalate had become exhausted, as it has been reported to be likely to happen [17], two consecutive extractions of 2 h with 20 ml of reagent were carried out. Results showed that the application of the second 2 h step increased the extracted arsenic by 20%. Taken into account that the arsenic leached in this fraction is notably higher than in the previous ones, two consecutive 2 h extractions with 20 ml of reagent are necessary.

4.1.3.2.6 As associated with poorly crystalline Fe (hydr)oxides: CBA

The mixture 0.2 M sodium citrate/0.6 M sodium bicarbonate/0.4 M ascorbic acid (pH= 8.0) proposed by Paul et al. (2009) [94] was adopted for the dissolution of this phase. Extraction times of 8, 16, 21 and 24 h with 40 ml of extracting agent were compared. The highest arsenic concentration was obtained after the extraction for 21 h. In addition double extraction with consecutive steps of 16, 21 and 24 h were essayed. From results it is clear that double extractions were more efficient than single extractions. However, similar results were obtained with the two consecutive steps of 21 and 24 h. Hence, the shorter extraction was preferred.

Since this mixture of reagents has not been frequently employed, further optimisation of this stage was considered necessary. In this sense, solid-to-solution ratio was increased by using 60 ml of extracting agent. As this condition did not provide a greater arsenic lixiviation, it was ruled out.

It should be remarked that the optimization process of this stage was carried out with a control sample containing a great amount of arsenic and two consecutive extractions were necessary. However, some samples whose arsenic concentrations were lower than 1000 $\mu g \cdot g^{-1}$ were submitted to the two consecutive 21 h extractions and the leached arsenic in the second one was negligible. Hence, it was adopted the criterion that only samples exceeding 1000 $\mu g \cdot g^{-1}$ of total arsenic content would need two consecutive 21 h extractions.

4.1.3.3 Sequential Extraction Procedure Assessment

Fig. 4.1.2 shows the results of arsenic fractionation after submitting the natural As-bearing minerals and the As-spiked phases to the whole sequential extraction scheme. They are normalised to 100% in the figure.

Arsenic recovery, as the sum of the arsenic leached in every extraction step, compared to the total arsenic concentration, ranged between 85 and 115% for the studied matrices. Results confirm that arsenic is mostly leached in every solid material with the chemical extractant intended to target the desired phase.

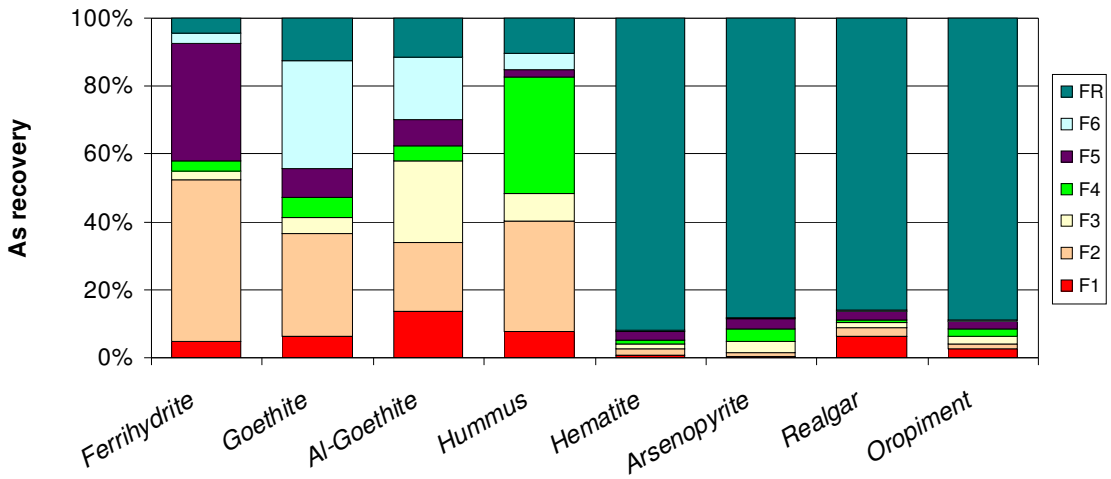

Fig. 4.1.2. Results of the SEP applied to natural and synthesized As-bearing phases.

An important part of the spiked arsenic in the matrices ferrihydrite, goethite, Al-goethite and hummus was adsorbed superficially onto their surfaces, since it was readily soluble in water (F1) or liberated by competitive desorption with phosphate (F2). This can be possibly linked with the preparation of these matrices, since some authors have suggested that the subsequent treatment of the mineral mixture (especially grinding) leads to a shift in the individual forms towards more labile arsenic bonding [97].

Another remarkable arsenic proportion was leached in the spiked ferrihydrite with the oxalate buffer (F5), which is completely in accordance to the amorphous nature of this oxyhydroxide.

As regards pure goethite and goethite partially substituted with Al, the main difference between both matrices lies in the fact that higher arsenic concentration was

extracted during the NH_4F step (F3) for Al-goethite (containing 11% Al) than for pure goethite (containing only 4.8% Al). This behaviour is expected since NH_4F extracts arsenic associated with Al oxyhydroxides. Likewise, a higher arsenic percentage was leached in F6 for pure goethite, as it corresponds to a weakly crystalline iron oxyhydroxide.

Arsenic fractionation in the spiked hummus shows that the largest arsenic concentration was extracted during pyrophosphate step (F4), which indicates an important association between spiked arsenic and humic matter. Moreover, significant Fe and Ca contents are extracted in F4 (Fig. 4.1.3), suggesting that arsenic species could have formed complexes with these cations bound to humic matter rather than directly with the organic functional groups.

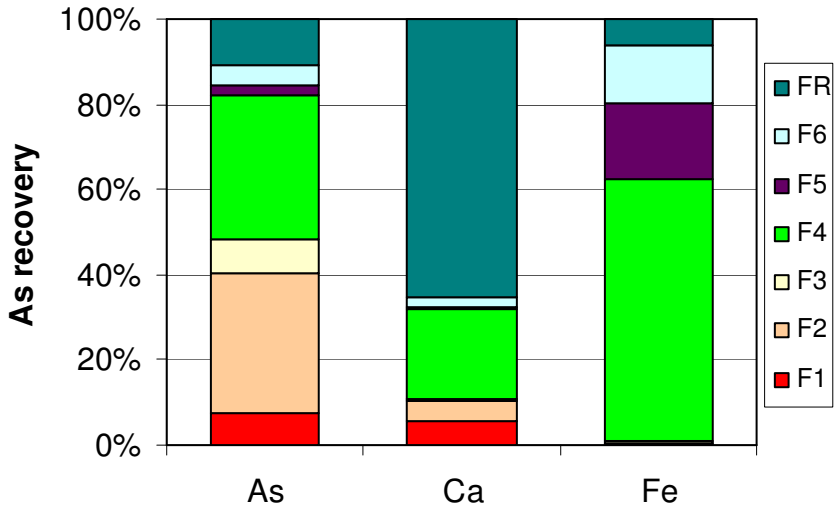

Fig. 4.1.3. As, Ca and Fe fractionations in the matrix arsenic-spiked hummus.

Most of the arsenic in the spiked hematite remained relegated to FR, indicating that arsenic coprecipitated with this mineral is not available at all and that very drastic conditions would be necessary to leach it.

As expected, the bulk of arsenic from minerals (arsenopyrite, realgar and orpiment) remains in the residual fraction. This means that arsenic in crystalline sulphides minerals is essentially immobile. However, around 10% of total arsenic content is leached in the previous steps, which can be attributed to the fact that these are natural minerals, not synthesized pure phases, and can contain little amounts of other more mobile arsenic-bearing minerals.

All these results reinforce the intention of the proposed SEP of distinguishing among the labile fractions and to relegate to the residual fraction all the minerals forms that hardly can be available.

4.1.3.4 Application of the SEP to solid environmental samples from different origins

Arsenic fractionation of 5 As-contaminated samples taken from different origin and with a wide range of arsenic contents was evaluated as an example of the usefulness provided by the proposed sequential extraction scheme. Two samples (S2 and S9) were taken from mercury mine areas, S9 subjected to acid mine drainage and S2 from carbonated soils. R1 came from arsenic mine. NIC was collected in a volcanic iron mine and CUB is a sediment sample with arsenic coming from an industrial origin. Total elemental composition and selected physico-chemical characteristics of the studied solid samples are given in Table 4.1.2. The fractionation results with the proposed SEP method are depicted in Fig. 4.1.4. Recovery of arsenic ranged from 85% to 104% in the studied samples.

Table 4.1.2. Physico-chemical characteristics and total elemental composition of the studied samples.

Sample	pH	Eh (mV)	TOC (%)	As ($mg \cdot kg^{-1}$)	Al (%)	Ca (%)	Fe (%)	Mn ($mg \cdot kg^{-1}$)
S2	7.91	102	7.4	11100	1.48	35.6	1.56	455
S9	2.15	487	2.2	11800	6.59	0.052	12.2	62
R1	7.30	111	9.4	67000	1.19	19.6	10.6	223
NIC	6.45	286	0.6	49	4.19	0.076	20.5	182
CUB	7.10	186	5.3	29	4.40	9.16	3.18	519

Arsenic profiles illustrate the variety of arsenic associations with the solid phases present at polluted sites and the dependency of arsenic mobility on the pollution source.

Sample S2 exhibited the highest arsenic mobility, as the percentages of arsenic leached in fractions F1 and F2 show (4.5% and 18.3% respectively). It means that more than 20% of arsenic in this sample can be considered available, as it is shown through its mobility to waters [98] and its bioavailability for plants [35]. The significant water-extractable arsenic can be attributed to the extraction of highly soluble arsenates, as Na and Ca arsenates [5], which is supported by the high Ca concentration in this sample (35.6%) and in the surroundings waters [98], On the other hand, the elevated Ca content, the highest of all the studied samples, is related to the

important presence of calcite in this mining area, as previous XRD studies have proved [57]. Arsenic adsorption onto calcium carbonates has been stated. They can provide sites for arsenic retention, especially calcite. Arsenate can interact specifically with surface structural Ca^{2+}, probably as outer sphere complexes [78, 99]. It is a loosely bound phase susceptible to adsorption–desorption reactions [100, 101]. Hence, arsenic bound to carbonates is expected to be released in step 2 by competitive desorption with phosphate anions. The greater percentage of arsenic extracted in F2 for sediment S2 than for the other samples can be attributed to the larger quantity of carbonates in this mine site. The hazardous potential of spoil heaps in this mining area must be considered, taking into account the high total arsenic content of this sample (11100 $mg \cdot kg^{-1}$) and the high mobility that it exhibits.

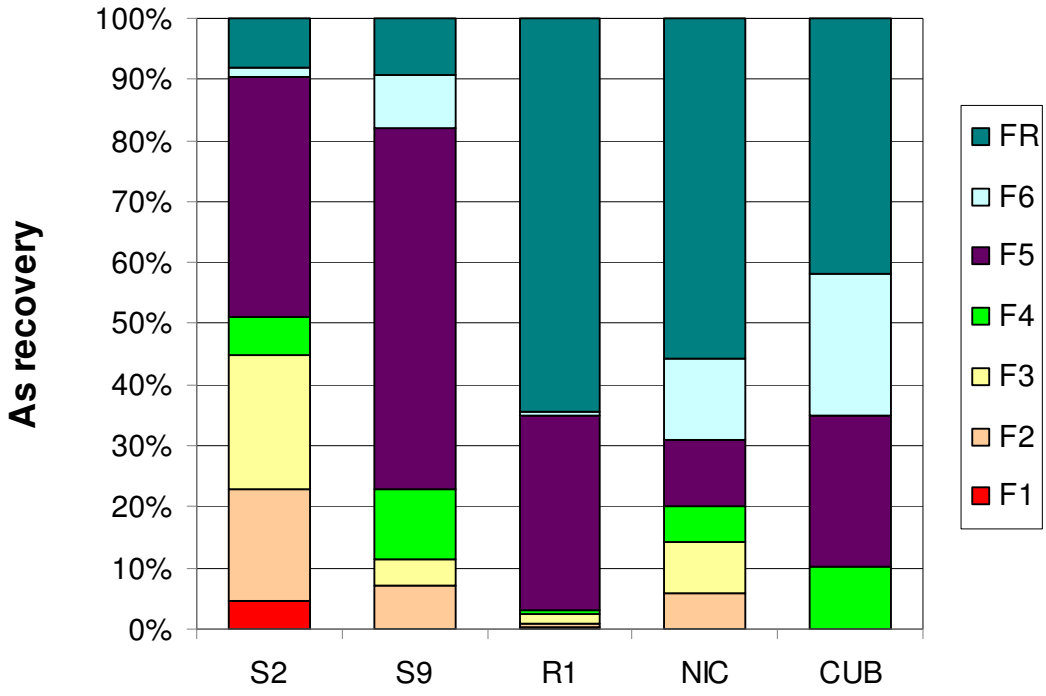

Fig. 4.1.4. Arsenic partitioning in the studied samples.

Arsenic appears mainly associated with Al and Fe oxyhydroxides in both samples S2 and S9, coming from cinnabar mines. The important arsenic extraction in fractions F3 and F5 is consistent with the origin of samples since they were collected in the surroundings of spoil heaps where large volumes of roasted rocks and mine-ore waste materials are present. These spoil heaps are characterized by its red-brown color due to the presence of abundant iron oxides as fine-grained particles. Retorting of Hg-bearing ore is known to be an inefficient and incomplete process, and as a consequence, sulphide minerals can be converted to higher mobile phases as sulphates, arsenates and oxyhydroxides [102]. The extent of arsenic association with

amorphous and weakly crystalline iron oxyhydroxides was markedly greater for S9 than for S2. This is the consequence of the higher presence of iron oxyhydroxides in S9, as it is evidenced by the higher total Fe concentration in this sample. Arsenic in the residual fraction did not reach 10% for none of the two samples, indicating that the intense mining activity carried out in these mines has led to the conversion of primary and refractory minerals into other more labile ones.

On the contrary, in the sample R1, coming from an arsenic mine, the residual fraction was dominant. F5 is also a very important fraction, and both F5 and FR represent more than 96% of total arsenic, This is in agreement with the fact that this mine has been less exploited than the previous ones and that primary minerals such as arsenopyrite, As-rich pyrite or realgar has been found in the spoil heaps in a low degree of alteration [103]. The leached arsenic in F5 may be the consequence of the ore extraction and smelting carried out in this site, that provokes partly conversion of primary minerals into others more labile, usually in amorphous forms [103].

Sample NIC corresponds to a geothermal active area where an unexploited mine exists. This sample presents an elevated percentage of arsenic in the residual fraction, according to its volcanic origin, since igneous rocks contain high proportion of arsenopyrite, As-rich pyrite, orpiment, realgar and base metal sulphides. The arsenic extracted in F5 and F6 corresponds to the Fe (hydr)oxides also present in this area [80, 104]. The significant arsenic percentages found in fractions F2 and F3 also indicate that weathering of As-bearing sulphides occurs, leading to more available soil arsenic phases. They are also the consequence of the contamination that this area suffers from the indiscriminate use of pesticides.

Sample CUB corresponds to a sediment collected in the Cienfuegos Bay (Cuba), surrounded by an industrial area where discharges of heavy metals and organic compounds were common in the past. Although Taggart et al. (2004) [105] reported that arsenic is present at high percentages in the more mobile fractions in anthropogenic contaminated soils, in this sediment from an aquatic environments the most labile forms are not present due to the leaching process occurred over time. In this case, the proportion of arsenic found in F1, F2 and F3 is negligible and the more labile arsenic forms are associated with organic matter (F4), as a consequence of the permanence of organic compounds in water over time. Similar results were found in this fraction in wet soils [106]. Given the low arsenic content of this sample and its low arsenic mobility, this sample does not involve an important environmental risk.

4.1.4 CONCLUSIONS

A novel sequential extraction procedure has been developed for arsenic partitioning in the most relevant phases present in environmental solid samples with emphasis in the most labile arsenic fractions. The arsenic fractions are: readily soluble, strongly adsorbed onto mineral surfaces, associated with Al oxyhydroxides, bound to organic matter, incorporated into amorphous Fe oxyhydroxides, associated with poorly crystalline Fe (hydr)oxides and coprecipitated with refractory minerals. Optimization experiments were made allowing the adequate arsenic extraction of each fraction in samples with both low and even extremely high arsenic contents. Assessment experiences with natural and synthetic As-containing minerals and As-spiked matrices proved that the proposed SEP is fairly selective since As-bearing phases were mainly leached by the reagents proposed to target those phases.

Application of the proposed SEP to real world samples of different origins, nature, sources of pollution and with a wide range of arsenic concentrations was carried out. The overall effectiveness of the proposed sequential extraction process was assured since arsenic recoveries ranged from 85 to 103% in all the studied samples, indicating that the method is appropriate for a great diversity of environmental solid samples.

Arsenic distribution profiles were consistent with the sources of arsenic pollution and the nature of samples which demonstrates the suitability of the proposed method to predict the potential of mobilization and bioavailability of arsenic.

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4.2 Comparison of three sequential extraction procedures for arsenic fractionation in highly polluted mining sediments

ABSTRACT

An evaluation of three sequential extraction schemes for the study arsenic fractionation in environmental solid samples was performed. The considered protocols were: i) the standardized and widely recognised BCR, conceived for the study of heavy metals partitioning, ii) the protocol developed by Manful (1992) as an adaptation from a phosphorus scheme for arsenic fractionation, and iii) a novel sequential extraction scheme especially devised for arsenic. The efficiency and suitability of these methods and their corresponding extraction steps to partition arsenic into the most important solid pools were tested by their application to some real sediment samples heavily polluted by mining activities. Results showed the inappropriate use of BCR scheme for arsenic fractionation Nevertheless, BCR protocol could provide a first approach for the assessment of arsenic partitioning, as its first extraction step can be considered adequate as an estimation of the most easily mobilizable arsenic. Although Manful scheme offers a more differentiated arsenic pattern, some drawbacks arise from the lack of selectivity provided by some of the employed reagents, as the overlapping of certain target phases, the overestimation of adsorbed arsenate in detriment to the importance of coprecipitation processes and the incapacity to discriminate among amorphous the crystalline oxyhydroxides, main responsible for arsenic retention. The proposed protocol achieves the most suitable arsenic fractionation, since the main phases retaining arsenic are selectively targeted according to its potential grade of mobilization. In addition, the simplicity of its extraction steps would allow the automation into a continuous flow scheme.

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4.2.1 INTRODUCTION

Arsenic is a harmful metalloid that is widely distributed in the earth's crust. Its distribution among geochemical sources is irregular. The mean value reported for uncontaminated soils is 5-10 $mg \cdot kg^{-1}$ [1], whereas natural values for sediments are below 10 $mg \cdot kg^{-1}$ [2]. Anthropogenic activities represent the major cause of arsenic pollution in the environment. The use of arsenical pesticides, combustion of fossil fuels, mining works and ore processing, or industrial waste dumping, make rise greatly the arsenic accumulation in soils and sediments [3].

Arsenic usually appears in soils and sediments forming oxyanions, being arsenite (As(III)) and arsenate (As(V)) the predominant forms [4]. In oxygen-rich environments and well-drained soils, arsenate species dominate ($H_2AsO_4^-$ in acidic soils and $HAsO_4^{2-}$ in alkaline ones) whereas under reducing conditions, such as regularly flooded soils, arsenite is more stable [5, 6]. Since the toxicity of an element is related to its mobility and availability, arsenites can be considered more toxic than arsenates because of its higher solubility [5].

Arsenic interactions with soil constituents are very diverse. The relative predominance of arsenic forms in soils depends on the type and abundance of sorbing components of the soil, the pH and the redox potential [7-9].

Arsenic sorption onto soil particles is one of the most important processes that immobilises this metalloid [10]. Arsenate and arsenite adsorb onto surfaces of a variety of materials, especially iron oxides and aluminium oxides [11]. The adsorption onto the edges of clay minerals or onto the surface of calcite has been also stated [12]. Adsorption and desorption processes between arsenic and iron-oxide surfaces are particularly important since iron oxides usually occurs as coatings on other solids [11]. It is well established that these reactions are mainly pH dependent. Arsenate is preferentially sorbed on hydrous oxides at pH values ranging from 4.0 to 7.0, whereas arsenite is preferentially sorbed at pH values from 7.0 to 10.0 [13]. Desorption of arsenate from Fe-oxide surfaces takes place at alkaline pH values [11, 14].

An important arsenic affinity for soil organic matter has been also reported, especially for humic acids (HA) and fulvic acids [15, 16] [17-20]. They may interfere strongly with arsenic adsorption under some circumstances and arsenic mobility may be increased by their presence in soil [21-23]. The interaction is postulated to involve bridging metals and deprotonated functional groups within the HA [19].

Coprecipitation of arsenic with iron oxyhydroxides has been defined as the major mechanism retaining arsenic, and this binding is particularly strong [24]. Arsenic-bearing iron oxyhydroxides may act as an arsenic source in the case of reductive dissolution of both amorphous and crystalline oxyhydroxides, being the former species more labile than the crystalline phases [25, 26]. The association of arsenic to hydrous aluminium and manganese oxides may also be important if they appear in significant quantity [27, 28], and they are more labile phases than the iron minerals [29].

Other more recalcitrant arsenic forms are the naturally occurring arsenic sulphide ores, being the most frequent arsenopyrite (FeAsS), arsenian ('As-rich') pyrite ($Fe(S,As)_2$), realgar (AsS) and orpiment (As_2S_3) [10, 30]. The presence of primary arsenic minerals in arsenic contaminated soils represents a long-term arsenic pollution source, since it has been hypothesized that weathering of sulphides led to the formation of other secondary forms, thermodynamically more stable and soluble [26, 31].

Sequential extraction procedures (SEP) can be used to determine the above mentioned binding fractions of elements in solid environmental samples. In this process, the sample is subjected to a series phase specific reagents of successive stronger dissolving power under controlled conditions which remove metals from the particular physicochemical phase of concern [32]. Depending upon fractions of interest, a broad range of chemical extractants can be used and thus in the literature numerous sequential extraction schemes are available, which vary in the use of extractant, target phase and the order of attack to separate a particular form of metals. The bulk of the schemes are deviations of the scheme proposed by Tessier et al. (1979) [33, 34]. Many researchers have reported difficulties in comparing the results of SEP due to their wide variation in the use of chemical reagents and target phase [35]. Thus, in an effort to synchronise the diverse methodologies and to facilitate the easier comparison of results, the Community Bureau of Reference (BCR), now the Standards, Measurement and Testing Programme, (SM&T) proposed a three step extraction procedure along with a reference sediment material to certify the protocol [36-41]. Although it was designed for heavy metals, it has been occasionally applied to evaluate arsenic fractionation [42-46].

Opposite to the cationic nature of the other trace metals, arsenic is predominantly present in soils and sediments as oxyanions. Hence, the traditional schemes have been not recommended for arsenic and protocols especially designed for its study have been advised [47]. Since arsenic characteristics resemble more the

phosphorus behavior, especially with respect to ion exchange, sequential extraction schemes specifically developed for phosphorus [48] have been adopted and modified for arsenic, as the scheme proposed by Manful (1992) [49].

The aim of the current research is the comparison of three sequential extraction schemes for the study of arsenic fractionation. The first protocol is the standardised and widely recognised BCR, conceived for the study of heavy metals partitioning. The second one is a consolidated protocol developed by Manful (1992) [49] adapted from a phosphorus scheme for arsenic fractionation. The third one is a novel sequential extraction scheme especially devised for arsenic. The efficiency and suitability of these methods and their corresponding extraction steps to partition arsenic bound to the most important solid pools were tested by application to some real sediment samples heavily polluted by mining activities.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Sites descriptions and sampling points

Samples were collected from two old abandoned mining districts located in Asturias, Northern Spain, where abundant mercury deposits existed and As-rich minerals occurred in paragenesis with mercury ores [50]. The presence of arsenic sulphides in the area is frequent in the form of orpiment, realgar and As-rich pyrite [51-53]. The cinnabar mines were exploited for years, but after the closures, they were abandoned without receiving any treatment to minimize the environmental impact and their corresponding spoil heaps, containing waste residues from ore processing operations with high concentrations of arsenic and other heavy metals, currently remain open-air. Natural weathering or variations in physicochemical parameters at the disposal sites can alter the stability of waste materials, mobilizing mercury, arsenic and related heavy metals to the soils and sediments [54]. The arsenic release from these solid environmental materials can reach the surrounding waters [55] and even plants [56].

Sediments from three points were sampled in **"**La Soterraña" mine: S1, collected under a pipe receiving runoff upstream of the waste piles. S2, located in an open-air channel, just downstream of the main spoil heaps and receiving runoff waters and their leachates. S3 corresponds to a stream downstream from S2, receiving also the aforementioned leachates. From "Los Rueldos" mine site two sediments were

taken, both of them affected by acid mine drainage (AMD): S8, collected in a pond outside the portal of the mine, and S9, situated a few meters from the previous one, inside the mine gallery.

4.2.2.2 Reagents, materials and apparatus

All the solid reagents employed in the preparation of the extracting reagents were of reagent grade quality and all the acids employed in the acid mineralization, HNO_3 (65% w/v), HCl (37.5% w/v), HF (48% w/v) and $HClO_4$ (70% w/v) were of ultrapure quality, provided by Merck (Darmstadt, Germany). Ultrapure water (Millipore, Bedford, MA) was employed throughout. All glassware and bottles were cleaned by soaking in 0.5 M HNO_3 for 2 days and rinsed three times with deionized water before use.

The equipment employed in the assistance of the extractions consisted on a vortex mixer (Labnet Inc.) with variable speed, a bench-top centrifuge (Eppendorf 5804), an end-by-end shaker (Bunsen ARR-8), a drying thermostatized oven (Proeti S.A.) with a maximum adjustable temperature of 200 ºC, automatic agate mortars (Fischer Scientific Co.) and an automatic homogenizer (Spex Mixer/Mill Cat. 8000). For the extraction experiments 50 ml polypropylene centrifuge tubes were used.

4.2.2.3 Total elemental analysis and physicochemical characterization of samples

Determinations of total elemental composition in the studied samples were carried out by acid digestions in microwave oven. 0.25 g of sample was weighed in Teflon vessels and a mixture of 7.5 ml of HNO_3, 2.5 ml of HCl and 6 ml of HF was added. They were digested in an Ethos One microwave system (Milestone, USA) at 220 ºC for 15 minutes. Resulting digests were transferred to Teflon capsules and 2 mL of $HClO_4$ were added and heated in a hot plate until evaporation of hydrofluoric acid, transferred into 25 ml volumetric flask, filtered through 0.45 µm cellulose paper when necessary, acidified with nitric acid to pH 2-4 and stored at 4 ºC until analysis. Appropriate dilutions were made when they were required. Validation of the procedure was performed with CRM NIST 2710 (Montana soil), a highly contaminated soil. Estimated errors were lower than 5% for all the studied elements.

Arsenic, aluminium, calcium, iron and manganese contents in the solutions resulting from every leached step, as well as in the solutions resulting from the total

acid digestions, were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a VARIAN 735-ES instrument with radial configuration following the EPA/200.7 method [57, 58]. Quantification limits, calculated as ten times the standard deviation of a blank injected 10 times divided by the sensitivity (slope of the calibration curve), were 5 $\mu g \cdot l^{-1}$ for Al and Ca and 3 $\mu g \cdot l^{-1}$ for As, Fe and Mn.

Sulphur contents were measured by elemental analysis by combustion, with a LECO CS-244 with infrared detection, following the EPA/9056A method [59]. The detection limit was 0.05% S.

Mercury analyses were carried out by using a direct mercury analyzer (DMA-80, Milestone). Determinations were made directly on solid matrices. Due to the extremely high mercury contents of sediments it was necessary to prepare dilutions over the solid samples with silica free of mercury according to the protocol of Fernández-Martínez [60]. The detection limit was 1 $\mu g \cdot kg^{-1}$. To ensure the quality of the results, the same procedure was applied to the CRM NIST 2711 (Montana soil), containing moderately elevated trace element concentrations, whose total Hg content is 6.25 ± 0.19 $mg \cdot kg^{-1}$ The obtained result was 6.10 ± 0.10 $mg \cdot kg^{-1}$, in good agreement with the certified value.

Total organic carbon was determined as NPOC (Non Purgeable Organic Carbon) with an analyzer TOC (Shimadzu–VCHS) by means of oxidative combustion and determination with a non-dispersive infrared sensor (NDIR). The detection limits for both of them are 12 $mg \cdot l^{-1}$.

pH and Eh of soils and sediments was measured with a Pt-Ag/AgCl electrode (CRISON) on sample/water suspensions at 1:2.5 ratio (w/v).

Characterization of crystalline phases of samples was carried out with an X-ray powder diffraction spectrometer (X'Pert PRO, PANalytical) with Cu K_α radiation at 45 kV and 40 mA. The detection limit for this technique is about 1%.

4.2.2.4 Sequential extraction schemes

The sequential extraction schemes here employed are summarized in Tables 4.2.1, 4.2.2 & 4.2.3.

Scheme I corresponds to the original BCR scheme [36-38]. It was slightly modified, since extraction stages last for 20 hours instead the established 16 hours.

Moreover, an extra residual step was added to determine the arsenic remaining bound to refractory minerals (Table 4.2.1).

Table 4.2.1. Extraction conditions for the scheme I: Slightly modified BCR protocol.

STEP	TARGET PHASE	EXTRACTION AGENT AND CONDITIONS
F1	Exchangeable, water and acid soluble As (bound to carbonates)	20 m 0.11 M CH_3COOH, 20 h
F2	Reducible species e.g. bound to Fe/Mn oxyhydroxides	20 ml 0.1 M $NH_2OH{\cdot}HCl$ (pH= 2), 20 h
F3	Oxidizable species e.g. bound to organic matter or sulphides	1) 5 ml 8.8 M H_2O_2 (pH= 2-3). Digestion in closed vessel at room temp, 1 h. Then at 85 ºC in water bath, 1 h. 2) 5 ml 8.8 M H_2O_2 (pH= 2-3). Digestion in closed vessel at 85 ºC, 1 h. Then digestion in open vessel at 85 ºC until near dryness. 3) 25 ml 1 M CH_3COONH_4, 20 h
FR	As coprecipitated with refractory minerals	Acid digestion in MW owen: mixture of 2.5 ml HCl + 7.5 ml HNO_3 + 6 ml HF

Table 4.2.2. Extraction conditions for the scheme II: Modified Manful protocol.

STEP	TARGET PHASE	EXTRACTION AGENT AND CONDITIONS
F1	Easily soluble As	30 ml H_2O, 12 h
F2	As associated with Al oxyhydroxides	30 ml 0.5 M NH_4F (pH= 8.2), 12 h
F3	Non occluded arsenate bound to the surface of Fe-rich minerals	0.1 M NaOH, 30 ml, 12 h
F4	As occluded within Fe/Mn-(hydr)oxides	CBD: 30 ml 0.5 M sodium citrate and 2.5 ml 1 M $NaHCO_3$ while adding 0.5 g $Na_2S_2O_4.2H_2O$, 15 min heating (85 ºC)
F5	Oxidizable species: bound to organic matter or labile As sulphides	20 ml 8.8 M H_2O_2 (to pH 2 with HNO_3); heat (85 ºC) until completely dry; redissolve with 30 ml 0.02 M HNO_3, 12 h
FR	As coprecipitated with refractory minerals	Acid digestion in MW owen: mixture of 2.5 ml HCl + 7.5 ml HNO_3 + 6 ml HF

Scheme II was taken from a modified version of the specific-arsenic fractionation scheme by Manful (1992) [49] proposed by Van Herreweghe et al. (2003) [5]. Some variations were also performed for this work. Firstly, the anion exchange membrane (AEM) strip step was removed, since authors reported no significant arsenic

leaching from highly contaminated samples [5]. Secondly, the residual step was changed to the acid digestion used for the other schemes (Table 4.2.2).

Scheme III is a novel sequential extraction scheme recently developed by the authors in CIEMAT to evaluate specifically arsenic fractionation in environmental solid samples [61] (Table 4.2.3).

Table 4.2.3. Extraction conditions for the scheme III: CIEMAT protocol.

STEP	TARGET PHASE	EXTRACTION AGENT AND CONDITIONS
F1	Readily soluble As	30 ml H_2O, 24 h
F2	Strongly adsorbed As onto mineral surfaces	40 ml 0.5 M Na_2HPO_4 (pH= 8), 8 h
F3	As associated with Al oxyhydroxides	30 ml 0.5 M NH_4F (pH= 8.2), 15 h
F4	As bound to organic matter	10 ml 0.1 M $Na_4P_2O_7$, 16 h
F5	As incorporated into amorphous Fe oxyhydroxides	20 ml 0.2 M ammonium oxalate/oxalic acid (pH= 3), dark, 2h + 2h
F6	As associated to poorly crystalline Fe hydr(oxides)	CBA: 0.2 M sodium citrate + 0.6 M sodium bicarbonate + 0.4 M ascorbic acid (pH= 8), 40 ml, 21 h (*)
FR	As coprecipitated with refractory minerals	Acid digestion in MW owen: mixture of 2.5 ml HCl + 7.5 ml HNO_3 + 6 ml HF

(*) This step should be made twice only for samples with As content > 1000mg·kg^{-1}.

All the reagents volumes were optimized for 0.5 g of sample. Solid samples were pulverised to a grain size < 63 µm fraction. Blanks were run simultaneously at all stages of the procedures and all extractions were performed in triplicate, so the expressed results are the mean values.

Extractions were conducted weighing 0.5 g of solid sample into 50 ml polypropylene centrifuge tubes and sequentially submitted to the different leaching steps. After the addition of the corresponding extracting reagent, the mixture was vortexed and mechanically agitated in an end-over-end rotatory shaker at 35 rpm during the established time for each step. Then, samples were centrifuged at 5000 rpm for 15 min. Supernatants were extracted with a Pasteur pipette and the residues were washed with 5 ml of ultrapure water (with the exception of the first stage in the case of

the second and the third schemes), vortexed, recentrifuged and separated again. The supernatants were combined, filtered through 0.45 µm cellulose paper, diluted with water in a 50 ml volumetric flask and refrigerated at 4 ºC until subsequent analysis. After previous steps, final residues were transferred to PTFE vessels and digested in a microwave oven to quantify the residual fraction.

4.2.3 RESULTS AND DISCUSSION

4.2.3.1 Physicochemical characterization of the studied samples and total elemental composition

A brief summary of samples characterization is presented in Table 4.2.4 and diffractograms of the studied samples in Fig. 4.2.1.

Samples from La Soterraña (S1, S2 and S3) exhibit neutral pHs and weakly oxidant redox potentials, typical of the calcareous soils from this area [62], and evidenced by the large calcium concentrations contained in these samples and the calcium minerals found by XRD, such as calcite ($CaCO_3$) or fluorite (CaF). Arsenic concentrations are very elevated, up to 11100 $mg \cdot kg^{-1}$, as well as mercury concentrations, up to 1330 $mg \cdot kg^{-1}$.

Table 4.2.4. Physicochemical characterization and total elemental composition of the studied samples.

	SOTERRAÑA			RUELDOS	
	S1	**S2**	**S3**	**S8**	**S9**
pH	7.35	7.91	7.46	2.16	2.15
Eh (mV)	173	102	80	448	487
TOC (%)	2.3	7.4	2.4	2.8	2.2
S (%)	0.37	0.39	0.14	1.6	2.0
As ($mg \cdot kg^{-1}$)	10800	11100	4770	30700	11800
Hg ($mg \cdot kg^{-1}$)	1330	126	121	151	9.03
Al (%)	7.3	1.5	6.8	5.1	6.6
Ca (%)	3.5	35.6	1.3	0.07	0.05
Fe (%)	4.5	1.6	4.0	6.4	12.2
Mn ($mg \cdot kg^{-1}$)	394	455	1400	31	62

The acid pHs and high Ehs in sediments from Los Rueldos (S8 and S9) prove the acid mine drainage occurring in this mine site. In addition, the high sulphur and iron contents in points S8 and S9 are indicative of the notably presence of pyrite and other sulphur minerals in the area. It is noteworthy the low calcium contents in these samples, suggesting the non-calcareous nature of this mine site. Likewise, Mn contents

are lower than in La Soterraña. Arsenic and mercury concentrations are also very high, up to 30720 and 151 mg·kg^{-1} respectively.

TOC and aluminium contents are in the same level in samples of both mine sites.

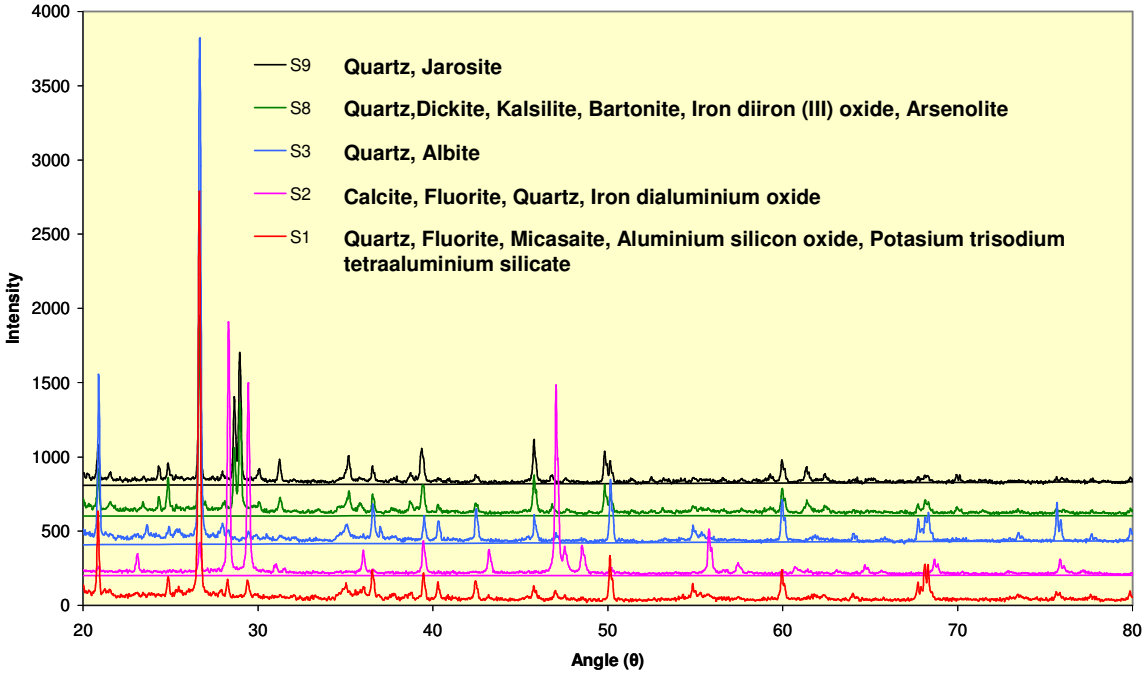

Fig. 4.2.1. X-Ray diffractograms of the studied samples.

4.2.3.2 Arsenic partitioning according to the employed sequential extraction schemes:

Results of the arsenic fractionation in all the studied samples are shown in Fig.4.2.2.

4.2.3.2.1 *Scheme I: Slightly modified BCR protocol*

Arsenic recovery rates were between 92.2 and 96.7% in all the studied samples, which can be considered acceptable. These values are slightly higher than the 89% obtained by Morillo et al. (2008) [63] for the reference material river sediment BCR CRM 320, but lower than the range up to 120% reported by Müller et al. (2007) [64] for natural highly polluted soils and sediments taken near abandoned tailings in Germany.

Although this protocol was designed for heavy metals, it has been occasionally applied to evaluate arsenic fractionation [42-46]. Therefore, an evaluation of the suitability of this protocol for arsenic study is necessary.

Clearly different distributions were obtained for samples from La Soterraña and Los Rueldos. Results are depicted in Fig. 4.2.2 A. The bulk of arsenic remains in the residual fraction in samples from Los Rueldos and it is distributed among the different steps in La Soterraña samples.

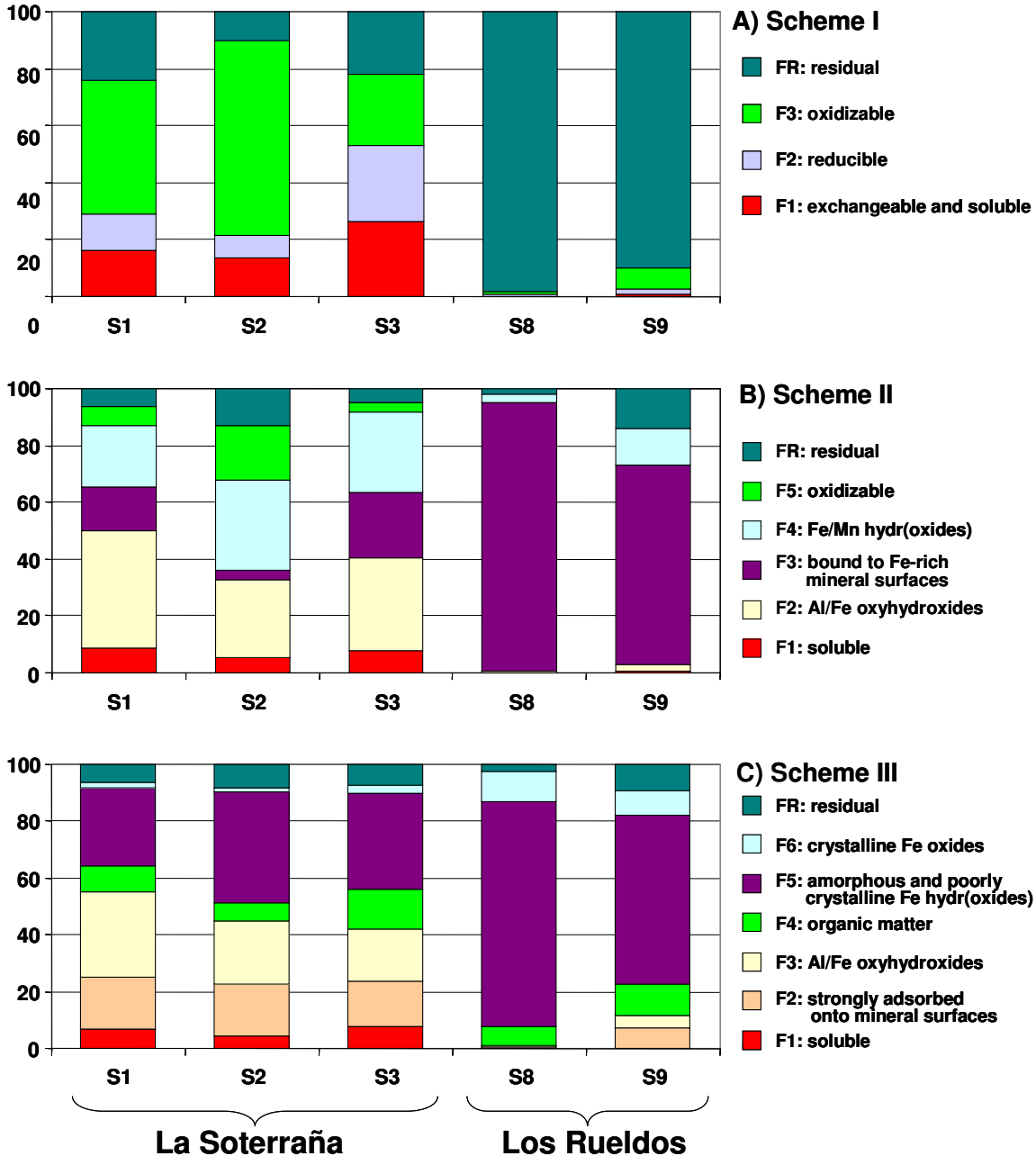

Fig. 4.2.2 Arsenic partitioning (%) in the studied samples with the followed sequential extraction protocols: A) Scheme I: Slightly modified BCR; B) Scheme II: modified Manful; C) Scheme III: CIEMAT.

The arsenic leached in F1 with this scheme is supposed to be bound to carbonates. It has been stated that calcium carbonates can provide sites for arsenic retention, especially calcite. Arsenate can interact specifically with surface structural Ca^{2+}, probably as outer sphere complexes. [26, 65]. In fact, some sequential schemes specific for arsenic have intended to target this phase by using acetate solutions [10, 66]. This fraction accounts around 20% in samples from La Soterraña. The elevated calcium concentrations in these samples seem to indicate the presence of calcite in this mining area, which is corroborated by the diffractogram of sediment sample S2 (Fig. 4.2.1). Due to the non-calcareous nature of Los Rueldos mining area, F1 is virtually insignificant for S8 and S9.

The percentages of arsenic leached in the reducible fraction ranged 7.3-25% for, samples from La Soterraña and almost negligible for samples from Los Rueldos. These results are totally different from those of Van Herreweghe et al. (2003) [5], since they found that arsenic was predominantly extracted in the reducible fraction of the BCR scheme both in highly polluted samples and in the reference materials Montana soil (SRM2710) and a coal fly ash (SRM1933b). However, our results are more in accordance with those of Müller et al. (2007) [64] as they barely extracted arsenic in the reducible fraction of BCR scheme in highly polluted samples because of mining. The low percentages found in reducible fraction were completely unexpected, since a vast majority of studies have established that aluminium, manganese and iron oxides and hydroxides are the main arsenic scavengers in environmental solid samples, especially the iron forms [67, 68]. The problem could be attributed to the inefficient dissolution of these phases by the hydroxylamine solution in the employed conditions. It is known that the dissolution of these hydr(oxides) increases with the increase of the reagent concentration, the agitation time, or lowering the pH [69]. In addition, iron extraction can be insufficient with this reagent for materials with a high Fe content [70, 71], as samples from Los Rueldos exhibit, and more aggressive conditions than those employed by this protocol would be required. This reagent has been included in certain arsenic-specific sequential protocols [10, 66]. However, in these cases stronger conditions were employed and the extraction of the reducible fraction even involved two consecutive steps of increasing strength. For instance, Kim et al. (2003) [66] firstly employed 25 ml of NH_2OH 0.1 M (pH 2) + KOH 0.1 M at room temperature for 20 hours for targeting only the easily reducible oxides, and afterwards they used the same mixture more concentrated (0.25 M of hydroxilamine) at higher temperature (50 ºC) for dissolving the amorphous oxide. Hall et al. (1996) [72] also used this reagent as a first step in mild extraction conditions to dissolve the arsenic bound to amorphous Fe-

oxyhydroxides and secondly they used more aggressive conditions to leach the arsenic bound to crystalline Fe-oxyhydroxides. Hence, the association of arsenic with hydr(oxides) is underestimated in BCR protocol because of the inefficient ability of the hydroxylamine under the employed conditions.

Conversely to the previous stage, the oxidizable arsenic fraction is overestimated by this scheme, since the bulk of arsenic in sediments from La Soterraña is extracted in F3 and these samples does not contain so important amount of organic matter or sulphides for being here released. The problem may lie in the lack of specificity of H_2O_2 extraction step. It is well known that Mn oxides are easily dissolved by H_2O_2 [73]. In addition, although iron oxides are in principle not removed by H_2O_2, its dissolution occurs if they are associated to manganese phases [74].

This method considers that nearly all the arsenic in samples from Los Rueldos is coprecipitated with refractory minerals, coinciding again with results of Müller et al. (2007) [64], who found more than 80% of arsenic in the residual phase. However, these samples contain important amounts of iron hydr(oxides) and sulphur minerals, as diffractograms of samples S8 and S9 show. It means that neither NH_2OH nor H_2O_2 have conveniently extracted the arsenic associated with oxyhydroxides. Moreover, given the incapacity of H_2O_2 to completely leach metals associated with sulphides [34], a great part of these minerals remain in the residual fraction.

4.2.3.2.2 *Scheme II: Modified Manful protocol*

Arsenic recovery ranged from 86.1 to 98.6% in all the studied samples.

This scheme offers a more differentiated pattern of arsenic distribution (Fig. 4.2.2 B) than the previous one since it intends to distinguish among important arsenic pools in solid samples.

The percentages of arsenic leached in F1 of this method are lower than those of the first step of BCR method. The water-extractable arsenic in F1 of the scheme II can be attributed to highly soluble arsenates, as Na and Ca arsenates [5]. However, in F1 of the scheme I, in addition to the readily soluble arsenic, the arsenic species bound to carbonates are also leached. Similarly to the scheme I results, this fraction is higher in samples from La Soterraña. The appreciable calcium concentrations of these sediments can involve the existence of calcium arsenates, since it has been stated that arsenates constitute the main secondary arsenic minerals [10]. However, the non-

calcareous origin of Los Rueldos mining site implies that the existence of calcium arsenates is not very important.

Fraction F2 considers the arsenic associated to Al oxyhydroxides. Since it has been reported that hydroxy-Al groups present on the external surfaces of micaceous minerals are especially significant in arsenic retention [75], this fraction is very common in arsenic-specific extraction protocols [5, 49, 76-79]. In all of them Al oxyhydroxides are invariably extracted by NH_4F solutions. From results, it is evident that samples from La Soterraña exhibit an important proportion of arsenic associated with aluminium oxyhydroxides, which does not occur in samples from Los Rueldos.

Although NaOH has been commonly employed in sequential protocols for leaching the pool of certain cations bound to organic matter [80-82], its use in specific protocols for arsenic intends the extraction of the arsenic fraction associated with iron minerals [69, 77, 78, 83]. NaOH is supposedly selective for arsenic adsorbed onto iron oxyhydroxides surfaces and do not extract arsenic coprecipitated with iron minerals [84]. According to Manful (1992) [49], NaOH extracts non occluded arsenate bound to the surface of Fe-rich minerals (F3). Since adsorption of arsenite and arsenate does not occur on (amorphous) iron (hydr)oxides at pH values higher than 10 [13], the employed NaOH solution provides an appropriate pH for the release of the adsorbed arsenic species.

The higher iron contents in samples from Los Rueldos entails a greater adsorption onto their minerals surfaces and therefore the arsenic leached with NaOH (F3) is higher in S8 and S9 than in the samples from La Soterraña. It is possible that aluminium arsenates are being extracted in this fraction, since they are soluble at pH >8 [5]. Conversely, iron arsenates can only be extracted under reducing conditions [5]. Likewise, Chang and Jackson (1957) [48] specified that compounds soluble under reducing conditions were not extracted in soda. However, in the practice, NaOH does not only extracts arsenic superficially adsorbed into iron minerals, but also part of the arsenic bound to organic matter, as it is evidenced by the dark colour of the NaOH extracts. This was also observed by Van Herreweghe et al. (2003) [5] when they applied the same stage of this scheme. Hence, this reagent can not be considered fairly selective for the pool it intends to target and another one would be preferred.

The fact that the mixture DCB extracts (F4) more arsenic from sediments from La Soterraña is unexpected, being their total iron concentrations lower than those from Los Rueldos. In addition, crystalline iron oxides are present in sample S8 in a large

extent (Fig. 4.2.1). The reason could be that this reagent is extracting the remaining iron arsenates not leached during the previous step, as well as it is reducing the amorphous iron oxyhydroxides. Another possibility is that DCB also leach the arsenic coprecipitated with Mn oxyhydroxides, and the presence of this element in La Soterraña mining site occurs in larger extent that in Los Rueldos. If a differentiation among amorphous and more crystalline iron phases is desired, the use of other selective reagent for amorphous iron minerals before this step is recommended.

Although the extraction of the oxidizable fraction with H_2O_2 in schemes I (F3) and II (F5) is performed in a similar way, the results disagree completely as a consequence of the differences between the previous steps of both methods. Nevertheless, analogously to results of scheme I, samples from La Soterraña present more arsenic in the oxidizable fraction, despite their lower contents of organic matter or sulphides than S8 or S9. Once again, the problem of this reagent consists on its inability to selectively extract arsenic bound to organic matter, and the partial extraction of organically-bound arsenic during the previous NaOH extraction. In addition, H_2O_2 does not completely leach metals associated with sulphides [34] and other phases may be co-extracted. This fact was corroborated by Mihaljevič et al. (2003) [85], since they extracted arsenic with H_2O_2 from a synthetic sample not containing any arsenic bound to sulphides or organic matter. Consequently, the selective extraction of the organically-bound arsenic fraction would be favoured with other more selective reagent.

4.2.3.2.3 *Scheme III: CIEMAT protocol*

Arsenic recovery ranged from 88.6 to 97.5% in all the studied samples.

The distribution profile resulting from the application of this method is shown in Fig. 4.2.2 C. It is unlike the other two ones, and particularly different from the scheme I. However, when arsenic fractionations by the schemes II and III are compared, the arsenic contents remaining in the residual phase are similar, which means that both have considered the same grade of arsenic mobilization, but the fractionation patterns differs because of the differences on several considered arsenic pools and the extracting agents.

The sum of the arsenic extracted in fractions F1 and F2 with the scheme III roughly coincides with the arsenic extracted in F1 following the scheme I. According to the literature the extraction of arsenates specifically bound to carbonates is expected to

occur in F2 in the scheme III by competitive desorption with phosphate anions [26, 65]. Therefore, the application of the proposed method results advantageous since it allows distinguishing between the immediately available arsenic fraction (readily soluble arsenic) and a potentially available fraction by competitive desorption processes (strongly adsorbed arsenic onto mineral surfaces).

Arsenic extraction with NH_4F occurs in larger extent in scheme II (F2) than in the scheme III (F3). This reagent supposedly leaches the arsenic superficially bound to aluminium (hydr)oxides by anionic exchange. A previous stage to leach other arsenic species strongly adsorbed onto mineral surfaces was not considered in the scheme II, as it does the scheme III by the phosphate extraction. It could be assumed that other arsenic species adsorbed onto mineral surfaces different from aluminium oxyhydroxides are extracted by NH_4F in scheme II. In addition, partial extraction of calcium and aluminium arsenates may occur [5].

As regards the organically-bound fraction, the arsenic extractions by the scheme II with H_2O_2 (F5) and by the scheme III with pyrophosphate (F4) are not equivalent. Scheme II is not recommended to quantify arsenic bound to organic matter, given the lack of selectivity of H_2O_2 as well as the partial dissolution of organic matter during the NaOH extraction,. On the contrary, 0.1 M pyrophosphate solution at pH 10 has been proved as a highly selective reagent to the extract humic acids fraction which are the main arsenic sorbent [17-19]. Hence, the proposed method results more suitable to adequately estimate the arsenic bound to humic acids fraction.

Extraction of arsenic coprecipitated with amorphous and crystalline iron (hydr)oxides is predominant in all samples (F5 and F6). This is consistent to the literature since co-precipitation with iron oxyhydroxides constitutes one of the main processes controlling the fate of arsenic in soils and sediments [29, 86]. In samples from Los Rueldos, arsenic extractions with NaOH from the scheme II (F3) and with oxalate from the scheme III (F5) are comparable (Fig. 4.2.2), which may lead to think that both extractants are equivalent. However, the conclusions derived from the application of the two methods are quite different. The scheme II attributes NaOH-extractable arsenic to non-occluded arsenate bound to the surface of Fe minerals whereas according to the proposed method (scheme III) oxalate extracts arsenic coprecipitated with amorphous Fe (hydr)oxides. Taking into account that adsorbed arsenates must be released during the phosphate and NH_4F steps as it has been demonstrated through optimisation studies [61]) and the selectivity of oxalate buffer to dissolve amorphous iron (hydr)oxides [87], it is evident that NaOH extraction is highly

unselective and may comprise a mix of adsorbed arsenates, arsenic bound to humic acids and arsenic coprecipitated with amorphous Fe (hydr)oxides. Moreover, for the scheme II coprecipitated arsenic is extracted with CBD from both amorphous and crystalline hydr(oxides). Hence, the scheme II is not able to discriminate between the different pools of iron hydr(oxides) retaining arsenic. Since these minerals are considered the more important arsenic scavengers in soils and sediments, a gradual extraction from the more labile associations to the most refractory ones is advisable. In consequence, the application of the scheme II tends to overestimate adsorbed arsenate as well as minimises the importance of coprecipitation processes.

On the other hand, CBD and CBA reagents theoretically should achieve the same grade of arsenic extraction from poorly crystalline forms, but the proportion of arsenic leached with CBD is markedly higher in samples from La Soterraña (Fig. 4.2.2). This can be attributed to the inefficient dissolution of the amorphous iron forms with NaOH from the scheme II compared with oxalate step from the proposed method. Optimisation studies of the proposed method demonstrated the necessity of a second extraction step to assure the entire dissolution of the amorphous Fe phase with the oxalate buffer [61]. The low percentages of arsenic extracted with CBA (10.8 and 8.4 % for S8 and S9 respectively) indicate that the iron (hydr)oxides which bind the bulk of arsenic may not to be crystalline. A similar conclusion was drawn by Müller et al. (2007) [64] when they applied the Wenzel method [88] to highly polluted samples by mining. This is because ore extraction and smelting usually provokes partly conversion of primary minerals into others more labile, usually in amorphous forms [89]. A priori it could led to think that this stage is not very important and that could be skipped. However, Müller et al. (2007) [64] found an important contribution of the crystalline phase for retaining arsenic (more than 45%) in the reference material river sediment GBW 07311, and more than 20% has been reported for a sediment with a little amount of arsenic concentration and whose source of pollution was industrial damping [61]. Hence, the association of arsenic with crystalline iron oxyhydroxides must be considered in sequential extraction schemes especially developed for it.

Since sulphide minerals are highly insoluble and can be considered refractory phases, the proposed method relegates its extraction to the residual fraction.

This proposed method could be easily automated because of the simplicity of its extraction steps. Conversely, the scheme II present some inconveniences that could difficult the automation of the method. For instance, some steps are performed at high temperature, as F4 and F5; a possible loss of sample can occur while adding H_2O_2

because of the violent reaction that can take place in samples with high organic matter content; and the addition little by little of the solid $Na_2S_2O_4.2H_2O$ from CBD mixture also displays an important drawback.

4.2.4 CONCLUSIONS

In general, the scheme I, slightly modified BCR, resulted not to be very suitable for the evaluation of arsenic partitioning in the studied samples. Despite fraction F1 can be considered adequate as an estimation of the most easily mobilizable arsenic fraction, this scheme underestimates the association of arsenic with iron minerals, since $NH_2OH{\cdot}HCl$ in the used conditions is unable of dissolving entirely the pool of As-bearing iron minerals. As a consequence, part of the unleached arsenic is extracted in the oxidizable fraction and the organically-bound arsenic is overestimated. As a result of the incapacity of H_2O_2 to completely leach the arsenic associated with refractory sulphide minerals, they were relegated to the residual fraction and this is also overestimated by this protocol. Nevertheless, it is undeniable the usefulness provided by this method for the fractionation of heavy metals in environmental solid samples, and it could be helpful as a first approach to assess the most labile arsenic when fractionation studies of heavy metals and arsenic are carried out together.

From the comparison of the scheme I and II (modified Manful protocol) it can be concluded that a protocol especially designed for arsenic achieves a better arsenic partitioning since it takes into account the anionic behaviour of this metalloid and the main soil phases retaining it. However, some drawbacks arise from the scheme II when it is contrasted with the CIEMAT protocol (the scheme III). On one hand, the lack of selectivity provided by some of the reagents employed, such as NH_4F, NaOH and H_2O_2. The use of NH_4F without any other previous step for the specifically adsorbed arsenic onto mineral surfaces entails that this reagent extracts other adsorbed species in addition to the arsenic bound to aluminium oxyhydroxides, and maybe also partially calcium and aluminium arsenates. NaOH extraction is highly unselective and may comprise a mix of adsorbed arsenates, arsenic bound to humic acids and arsenic coprecipitated with amorphous Fe (hydr)oxides. Hence, the application of the scheme II tends to overestimate adsorbed arsenate as well as minimises the importance of coprecipitation processes to retain arsenic species. Lack of selectivity can be attributed also to the use of H_2O_2 as regards organic matter extraction. On the other hand, NaOH and DCB overlap in the extraction of iron oxyhydroxides and the scheme II is not able to discriminate the amorphous forms from the crystalline ones. Since these minerals

are considered the more important arsenic scavengers in soils and sediments, a gradual extraction from the more labile associations to the most refractory ones is advisable.

The CIEMAT protocol offers the most differentiated pattern of arsenic fractionation, due to the careful selection of the main soil pools scavenging arsenic and its selective extraction as a function of its grade of lability. Thus, the consecutive extraction of the arsenic readily soluble, the specifically adsorbed onto mineral surfaces and the fraction superficially bound to aluminium oxyhydroxides rightly accounts the most mobilizable arsenic pools. The selective extraction of the organically bound arsenic is get due to the high affinity of pyrophosphate for humic acids. An adequate differentiation among the amorphous and crystalline iron phases is obtained with this protocol. Likewise, highly insoluble arsenic-sulphide minerals are relegated to the residual phase.

Finally, the simplicity of its extraction steps allows the automation into a continuous flow sequential extraction scheme which it will be addressed in the immediate future.

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4.3 Arsenic pollution and fractionation in sediments and mine wastes samples from different mine sites

ABSTRACT

A characterization of arsenic pollution and its associations with solid mineral phases in sediments and spoil heap samples from four different abandoned mines from Spain is performed. Three of them are mercury mines located in the same mining district, in the province of Asturias, and the other one, devoted to arsenic mining, in the province of León. For the study of arsenic partitioning, a sequential extraction procedure especially developed for arsenic was applied. Sampled sediments and mine wastes exhibited very notably arsenic concentrations, mostly above 1%. Arsenic fractionation in each mine is largely in accordance with the mineralogy of the area (soils and rocks) and the extent of the mine workings. In practically all the studied samples, arsenic appeared predominantly associated with iron oxyhydroxides, especially in the amorphous form. Sediments from cinnabar roasted piles showed higher arsenic mobility as a consequence of an intense ore treatment, posing an evident risk of arsenic spread to the surroundings. Samples belonging to wastes piles where the mining activity was less intense presented a higher proportion of arsenic associated with structural minerals. Nevertheless, it represents a long-term source of arsenic to the environment.

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4.3.1 INTRODUCTION

Arsenic is a ubiquitous element widespread in the environment. Its presence in soils can be related to the geological substratum, which implies that a wide range of arsenic levels can be found in uncontaminated soils, with a geochemical background of 5-10 $mg \cdot kg^{-1}$ [1]. For sediments, the natural values have been reported below 10 $mg \cdot kg^{-1}$ and also vary considerably all over the world [2]. However, anthropogenic activities such as the use of arsenical pesticides, combustion of fossil fuels, mining works and ore processing, or waste discharge of metal processing plants have often led to a greater accumulation of arsenic in soils and sediments [3].

Human exposure to arsenic polluted environments entails a health risk. Ingestion or inhalation of arsenic bearing particles represents a potential hazard [4], but of the various sources of arsenic in the environment, drinking water probably poses the greatest threat to human health [5]. Anyway, the biological availability and the physiological and toxicological effects of arsenic depend on its chemical form [6], the route and dose of exposure, and the susceptibilities of the tissues of each organism [7].

Arsenic usually occurs in soils and sediments as oxyanions in the oxidation states (III) and (V) [8]. In oxygen-rich environments and well-drained soils, arsenate species dominate ($H_2AsO_4^-$ in acidic soils and $HAsO_4^{2-}$ in alkaline ones) whereas under reducing conditions, such as regularly flooded soils, arsenite is more stable [9]. It is widely recognized that As(V) is less toxic and also less mobile than As(III), up to 10 times [9], since arsenate is adsorbed more strongly than arsenite onto mineral surfaces [10].

Mining and related works create tailings and leftover materials that can contain a great variety of arsenic compounds. Over 200 different mineral arsenic forms can be present in natural soils, of which approximately 60% are arsenates, 20% sulphides and sulphosalts and the remaining 20% includes arsenides, arsenites, oxides, silicates and elemental arsenic (As), but only some of them are commonly encountered [11]. The most important arsenic ores are arsenopyrite (FeAsS), arsenian ('As-rich') pyrite ($Fe(S,As)_2$), realgar (AsS) and orpiment (As_2S_3) [4, 12, 13]. It has been reported that in arsenic contaminated soils the weathering of primary arsenic minerals leads to the formation of other secondary forms, thermodynamically more stable. This fact can occur either through the adsorption of arsenic onto colloid surfaces, or by direct precipitation of arsenic solid phases [14, 15]. The relative predominance of arsenic forms in soils depends on the type and abundance of sorbing components of the soil,

the pH and the redox potential. As a general rule, the main arsenic secondary minerals present in soils and sediments are arsenates [4, 16]. The natural precipitation of hornesite ($Mg_3(AsO_4)_2.8H_2O$) [17], calcium arsenates and calcium-magnesium arsenates in lesser extent, aluminium arsenates and scorodite ($FeAsO_4.2H_2O$) [18] have been reported in mine wastes. Iron and aluminium arsenates are the dominant phases in acid soils and are less soluble than calcium arsenates, which predominates in alkaline and calcareous soils [19].

High arsenic concentrations are also found in many oxide minerals and hydrous metal oxides, either as part of the mineral structure or as sorbed species. Very remarkable are the concentrations that arsenic can reach in iron oxides, especially when they are formed by the oxidation of primary iron sulphide minerals containing arsenic. Hence, iron oxyhydroxides are the main mineral phase binding arsenic and this binding is particularly strong [20]. The association of arsenic to hydrous aluminium and manganese oxides may also be important if they have a significant presence [21, 22], as well as onto clay sorbents and onto the calcite surface [23]. Some authors have also considered that arsenic poses rather high affinity for soil organic matter [24, 25], especially for humic acids (HA) [26-29]. Arsenic mobility may be increased by its presence in soil [30-32]. The interaction is postulated to involve bridging metals and deprotonated functional groups within the HA. The association is dependent on pH, ionic strength and arsenic concentration. It has been reported that the extent of the interaction is greater in the 8-10 pH range for As(V) while it is extended to pH 12 for As(III) [28].

For assessing the mobility and availability of an element in the environment it is indispensable knowing the element distribution among the solid phases in soils or sediments, which is known as “fractionation”, following the recommendation of the IUPAC [33]. In this sense, sequential extraction protocols have been very useful, as well as widely employed and recognized. They are based on the sequential submission of the studied sample to selective reagents of increasing strengths to solubilise or extract the mineralogical phases responsible for retaining the larger part of the studied element. Extraction steps are intended to correspond with changes in the environmental conditions that could have effect on metal binding, such as acidification, oxidation or reduction [34]. From 1979 with the first sequential extraction scheme [35], most of them have been conceived for the partitioning of heavy metals. However, the behaviour of arsenic differs completely from them, since it is a semimetal present in soils and sediments usually as oxyanions, opposite to the cationic nature of the other

trace metals. Hence, the traditional schemes are not recommended when employed for arsenic, and protocols especially designed for its study are preferred [36].

In Spain, Asturias is a region with an old mining tradition. In fact, the most important mercury mining district of Spain behind Almadén is located there [37]. An intense mining activity was developed out during decades, until the 1970s when, because of the great decline on mercury prices most mines were closed [38]. In the most cases any restoration program was carried out after the closures, leaving behind the mine facilities and their corresponding spoil heaps abandoned, entailing an evident risk. Asturian cinnabar deposits are linked to As-rich minerals [39]. Hence, natural weathering or variations in physicochemical parameters at the disposal site can alter the stability of waste materials, including the As-rich mineral phases, releasing and mobilizing mercury, arsenic and related heavy metals to the surrounding soils and sediments, even to surface waters [40-42]. Likewise, in the Spanish province of León, an arsenic mine was also abandoned after intermittent exploitation in the mid XX century. The waste material remains currently untreated in two spoil heaps, posing a potential hazard related to arsenic mobilization [43].

Hence, understanding factors controlling arsenic mobilization may help to assess environmental impact and to make decisions regarding site remediation or encapsulation of spoil heaps [44, 45]. The objective of the current research is the study of arsenic fractionation in sediments and spoil heaps materials coming from four different mining areas by the application of a novel sequential extraction protocol especially designed for arsenic [46]. Arsenic partitioning is intended to be understood in terms of its relation with the mineralogy of the deposit in every case. Hence, differences in arsenic distributions and mobility among the studied mining areas are also evaluated.

4.3.2 MATERIALS AND METHODS

4.3.2.1 Sites descriptions and sampling points

Three of the four studied areas are mercury mines from Asturias (NW Spain): La Soterraña, located in the vicinity of the village of Pola de Lena, and La Peña-El Terronal and Los Rueldos, located close to Mieres.

La Soterraña mine exploited an epigenetic-type ore deposit, originated from low-temperature hydrothermal solutions [47]. The paragenesis of the ore deposit is mainly

constituted by cinnabar, orpiment, realgar, pyrite (usually with high arsenic concentrations), arsenopyrite, marcasite, and pararealgar, in a gangue of quartz and calcite. In mine wastes, iron sulphides (pyrite, marcasite and pyrrhotite) are quantitatively the most important metallic minerals phases, which decompose in the spoil heap, and breakdown of these minerals dominates the geochemistry of the spoil heap leachates. They are stable under reducing conditions, but they become unstable under oxidizing conditions. These abundant sulphides are typically in an advanced state of oxidation, with amorphous iron oxyhydroxides coatings, being also abundant crystalline mineral phases (dominated by goethite). Some relicts of cinnabar, arsenopyrite and sphalerite have been also found. The presence of realgar is frequent. Arsenic content of As-rich pyrites ranges from 0.11 to 4.93% and high arsenic concentrations have been also found in iron oxides, such as goethite (up to 2%). In this mine, sediments from three points were sampled (Fig. 4.3.1 A): S1, collected under a pipe receiving runoff upstream of the waste piles; S2, located in an open-air channel, just downstream of the main spoil heaps and receiving runoff waters and their leachates; S3 corresponds to a stream downstream from S2, receiving also the aforementioned leachates.

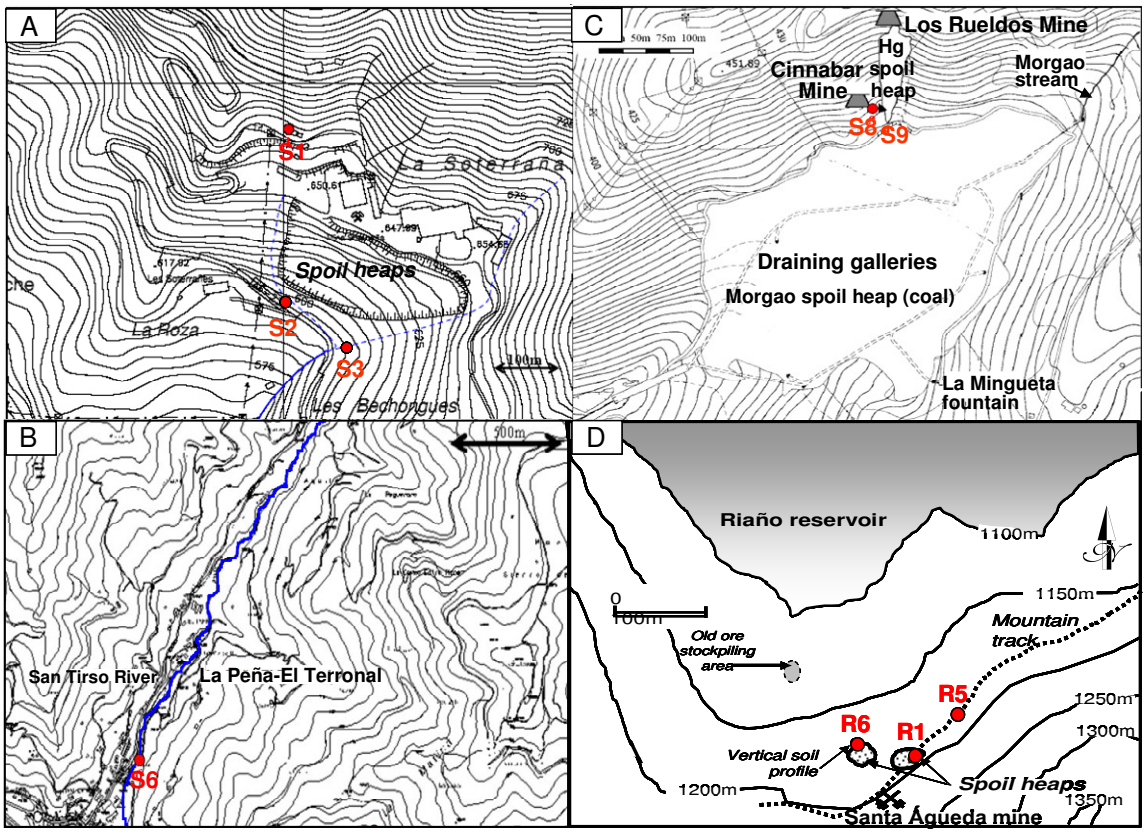


Fig. 4.3.1. Map of the four studied mines and sampling points: A) La Soterraña; B) La Peña-El Terronal; C) Los Rueldos; D) Santa Águeda.

In La Peña-El Terronal mine the presence of mercury is generally in the form of cinnabar, although metacinnabar and native mercury are often found [48]. Other present metallic minerals in the paragenesis of the ore deposits are pyrite, sphalerite, marcasite, chalcopyrite, galena, stibine, realgar and, exceptionally native gold [49]. Arsenic is usually present in the paragenesis of the ore deposits as orpiment and realgar, and more frequently associated with pyrite. The presence of arsenopyrite is scanty and limited only to some deposits. Regarding the subjacent substrate, the sandy mineral fraction (according to the Udenn-Wentworth grain size scale) is dominant in these soils [50]. Contrary to the other two mercury mines, where the closures were carried out without any restoration program, in this case a remediation plan consisting of the encapsulation of the main spoil heap was performed in 2002, when 300,000 tons of spoil heap wastes (constituted by tailings and granulated materials of various sizes) were isolated in an on-site security landfill [51]. The sample collected here, S6, is a sediment from San Tirso River, downstream of the mining facilities and the encapsulated spoil heap (Fig. 4.3.1 B).

In Los Rueldos mine the epigenetic deposit contains mercury mainly as cinnabar. Metacinnabar and native mercury are also occasionally found. Other primary metallic minerals which are present in the paragenesis of the ore are pyrite, occasionally As-rich pyrite, melnikovite, sphalerite, marcasite, chalcopyrite, arsenopyrite, galena, stibnite and realgar. Smithsonite, hemimorphite, cerusite, goethite, malachite, jarosite, melanterite, scorodite and gypsum are present as secondary minerals [52]. The gangue constituents are quartz, carbonates (calcite, dolomite and ankerite) and clay minerals (kaolinite and dickite). The ore deposit, from its geological, mineralogical and metallogenic characteristics, can be considered epigenetic, formed by the circulation of low-temperature hydrothermal solutions along distensional fractures [47]. Previous microscopic studies of representative samples from the spoil heap showed that iron sulphides (pyrite, marcasite and pyrrhotite) and iron sulphoarsenides (arsenopyrite) are quite abundant among the mine wastes stored in the spoil heap. Jamesonite replacing early sulphides is also frequent. Some relicts of cinnabar and sphalerite have also been found between the oxidized minerals on the studied samples. These sulphide minerals are typically in an advanced state of oxidation, with amorphous iron oxyhydroxide coatings. Scorodite is quite abundant too. Secondary gypsum is characterized by its relatively coarse grain size, with individual millimetric crystals [52]. At Los Rueldos mine site, two sediment samples were taken (Fig. 4.3.1 C), both of them affected by acid mine drainage (AMD): S8, collected in a

natural pond where AMD coming from the mining works is stored and S9, located a few meters from the previous one, inside the mine main gallery (10 m from its entrance).

Santa Águeda was a mine dedicated to arsenic extraction and processing located close to Riaño, at a topographic level of 1200 m, in the Northeast of León Province, in Spain. It was intermittently exploited since the nineteenth century, but nowadays it is abandoned. Since the abandonment, materials constituted by low-grade ore, enclosing rocks and smelting wastes remain disposed on land, in two spoil heaps at the mine site, inducing modifications of the landscape. An important aspect of this site is that Riaño and other small villages in the valleys of the upper catchments of the local Esla, Yuso and Orza Rivers have been flooded to construct a water reservoir for electricity generation and water supply for irrigation. Currently, sport fishing activities are carried out in this reservoir. The southern border of the dam is situated at about 300 m downstream of the mine works and spoil heaps, and mine drainage and spoil heap leachates consequently flow from the mined area to the reservoir. In this mine, the ore is constituted by arsenopyrite, As-rich pyrite, marcasite, stibnite, bravoite, realgar, scorodite, Sb-ochres, and goethite, within a gangue of quartz and carbonate. The mineralization appears as a fine-grained dissemination in a quartz-carbonated matrix constituted by jasperoid or millonitic to cataclastic fault breccias. Associated with the mineralization, there is an important silicification of carbonates and argillitization with formation of kaolinite on fissure planes [53, 54]. Materials stored in spoil heaps have a variable composition and heterogeneous distribution. Macroscopically, arsenopyrite and pyrite are very abundant. Supergenic minerals (scorodite, Sb-ochre, goethite and pararealgar) are also frequent among the mine wastes. Mineralogical studies show that arsenopyrite and pyrite are present in the spoil heaps with a weak-moderate weathering. Marcasite, bravoite, sphalerite, and cinnabar appear occasionally as relicts in pyrite and arsenopyrite crystals in some samples [43]. R1 and R6 were collected from the waste piles, R1 from the upper spoil heap and R6 from the lower one. R5 is a soil sample taken in the path to the spoil heaps (Fig. 4.3.1 D)**.**

4.3.2.2 Reagents, materials and apparatus

Nitric acid (65% w/v), hydrochloric acid (37.5% w/v), hydrofluoric acid (48% w/v), perchloric acid (70% w/v) and all solid reagents used in the sequential extraction applications were of analytical reagent grade, acquired from Merck (Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA) was employed for preparing all extracting solutions. All glassware and bottles were cleaned by soaking in 0.5 M HNO_3

for 2 days and rinsed three times with deionised water. For the extraction experiments 50 ml polypropylene centrifuge tubes were used.

Other equipment employed in the assistance of the extractions consisted of a vortex mixer (LabNet Inc.) with variable speed, a bench-top centrifuge (Eppendorf 5804), and end-by-end shaker (Bunsen ARR-8), a drying thermostatized oven (Proeti S.A.) with a maximum adjustable temperature of 200 ºC, automatic agate mortars (Fischer Scientific Co.) and an automatic homogenizer (Spex Mixer/Mill Cat. 8000).

4.3.2.3 Sampling

Sediments were taken from the upper 25 cm, between 1.5 and 2 kg per sample if possible, and introduced in a polyethylene bag. Once in the laboratory, samples were disaggregated and the non-mineral material and rock fragments higher 2 mm were discarded. Finally, they were oven dried at a temperature below 40 ºC to minimize loss of volatile elements (7-15 days), sieved and ground in an agate mortar to a size finer than 63 µm.

4.3.2.4 Physicochemical characterization and total elemental analysis

pH and redox potential (Eh) of solid samples were measured with a Pt-Ag/AgCl selective electrode (CRISON) on sample/water suspensions at 1:2.5 ratio (w/v).

Total organic carbon was determined as NPOC (Non Purgeable Organic Carbon) with an analyzer TOC (Shimadzu–VCHS) by means of oxidative combustion and determination with a non-dispersive infrared sensor.

Total acid digestions in samples were performed by an Ethos One microwave system (Milestone, USA). 0.25 g of sample was accurately weighed in Teflon vessels, a mixture of 7.5 ml of HNO_3, 2.5 ml of HCl and 6 ml of HF was added, and then they were digested at 220 ºC for 15 minutes. Resulting digests were transferred to Teflon capsules and 2 ml of $HClO_4$ were added and heated in a hot plate until evaporation of hydrofluoric acid, transferred into 25-ml volumetric flask, diluted with water to the mark, filtering when necessary through 0.45 µm cellulose paper and analyzed by ICP-AES. Appropriate dilutions were made for the analysis. Validation of the procedure was performed with CRM NIST 2710 (Montana soil), a highly contaminated soil. Estimated errors were lower than 5% for all the studied elements.

Determinations of arsenic, aluminium, calcium, iron and manganese in the different extracting solutions resulting from the application of every operationally defined phase to samples of study, as well as in the solutions resulting from the acid digestions, were analyzed by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a VARIAN 735-ES instrument with radial configuration following the EPA/200.7 method [55, 56]. Previously, they were acidified with nitric acid to pH 2-4 when it was necessary and stored at 4ºC until analysis. Quantification limits, calculated as ten times the standard deviation of the intensity of a blank introduced 10 times divided by the sensitivity (slope of the calibration curve), were 5 $\mu g \cdot l^{-1}$ for Al and Ca and 3 $\mu g \cdot l^{-1}$ for As, Fe and Mn.

Sulphur contents were measured by elemental analysis by combustion, with a LECO CS-244 with infrared detection, following the EPA/9056A method [57]. The detection limit was 0.05% S.

Mercury analyses were carried out by using a direct mercury analyzer (DMA-80, Milestone). Determinations were made directly on solid samples. Due to the extremely high mercury contents of sediments it was necessary to prepare dilutions over the solid samples with silica according to the protocol of Fernández-Martínez (2006) [58]. The detection limit was 1 $\mu g \cdot kg^{-1}$. To ensure the quality of the results, the same procedure was applied to the CRM NIST 2711 (Montana soil), containing moderately elevated trace element concentrations, whose total mercury content is 6.25 ± 0.19 $mg \cdot kg^{-1}$ The obtained result was 6.10 ± 0.10 $mg \cdot kg^{-1}$, in good agreement with the certified value.

Morphological analyses were performed by Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy (SEM-EDAX) by means of an instrument MEB JEOL-6100, which operates with a theoretical resolution of 4 nm.

4.3.2.5 Sequential extraction method

The selected sequential extraction procedure is the proposed by Larios et al. (2010) [46] and it is summarized in Table 4.3.1. 0.5 g of every solid sample was accurately weighed into 50 ml polypropylene centrifuge tubes and the corresponding extraction reagents were added sequentially. The mixture was vortexed and mechanical agitation in an end-over-end rotatory shaker at 35 rpm was maintained during each leaching step. After the extractions, samples were centrifuged at 5000 rpm for 15 min. Supernatants were extracted by using a Pasteur pipette and the residues were washed with 5 ml of ultrapure water (excepting for the first stage), vortexed,

recentrifuged and separated again. The supernatants were combined, filtered through 0.45 µm cellulose paper and diluted with water to a final volume of 50 ml. Finally, an acid digestion of the remaining residue was carried out following the same protocol that was used for total acid digestion with the conditions depicted in Table 4.3.1.

Table 4.3.1. Sequential extraction procedure.

Step	Target phase	Extractant
F1	Readily soluble As	H_2O, 30 ml, 24 h
F2	Strongly adsorbed As onto mineral surfaces	0.5 M Na_2HPO_4 (pH= 8), 40 ml, 8 h
F3	As associated with Al oxyhydroxides	0.5 M NH_4F (pH= 8.2), 30 ml, 15 h
F4	As bound to organic matter	0.1 M $Na_4P_2O_7$, 10 ml, 16 h
F5	As incorporated into amorphous Fe oxyhydroxides	0.2 M ammonium oxalate/oxalic acid (pH =3), dark, 20 ml, 2h + 2h
F6	As associated with poorly crystalline Fe (hydr)oxides	0.2 M sodium citrate + 0.6 M sodium bicarbonate + 0.4 M ascorbic acid (pH=8), 40ml, 21 h (*)
FR	As coprecipitated with refractory minerals	acid digestion in MW owen: mixture of 2.5 ml HCl + 7.5 ml HNO_3 + 6 ml HF

Every extracting volume is referred to 0.5 g of solid sample.
(*)This step should be made twice only for samples with As content >1000 $mg \cdot kg^{-1}$.

4.3.3 RESULTS AND DISCUSSION

4.3.3.1 Physicochemical characteristics of the studied samples and total elemental composition

Obtained results of the studied physicochemical parameters of samples, as well as results of total composition of some interesting elements are summarized in Table 4.3.2.

Table 4.3.2. Physicochemical characterization and total elemental composition of studied samples.

	Soterraña			Peña-Terronal	Rueldos		Riaño		
	S1	S2	S3	S6	S8	S9	R1	R5	R6
pH	7.35	7.91	7.46	6.90	2.16	2.15	7.30	7.15	6.96
Eh (mV)	173	102	80	90	448	487	111	156	141
TOC (%)	2.3	7.4	2.4	1.6	2.8	2.2	9.4	1.1	2.5
S (%)	0.37	0.39	0.14	0.09	1.6	2.0	4.2	0.05	0.08
As ($mg \cdot kg^{-1}$)	10800	11100	4770	310	30700	11800	67000	455	1060
Hg ($mg \cdot kg^{-1}$)	1330	126	121	1.87	151	9.03	56.9	1.33	4.53
Al (%)	7.3	1.5	6.8	3.9	5.1	6.6	1.2	6.5	4.3
Ca (%)	3.5	35.6	1.3	3.0	0.07	0.05	19.6	0.45	0.50
Fe (%)	4.5	1.6	4.0	2.4	6.4	12.2	10.6	3.7	3.6
Mn ($mg \cdot kg^{-1}$)	394	455	1400	377	31	62	223	300	598

4.3.3.1.1 *La Soterraña*

Samples from La Soterraña exhibit slightly basic pHs, typically of the calcareous soils present in this area [59], which is in agreement with the appreciable amounts of calcium found in these samples. Likewise, redox potentials of these sediments are weakly oxidant, which implies that AMD is a phenomenon that does not occur in this mine site.

Arsenic and mercury concentrations in S1 are very remarkable, 10800 and 1330 $mg \cdot kg^{-1}$ respectively. Arsenic content in this point is lower than those reported by Nagorski and Moore [60] for tailing-contaminated soils in Montana (up to 1100 $mg \cdot kg^{-1}$), but in the same range (up to 52600 $mg \cdot kg^{-1}$) than those reported by Kavanagh et al. (1997) [61] in soils and tailings-contaminated soils from UK. These high concentrations may be related to a mineralization outcrop, since this point is located upstream of the spoil heaps and the mining works. The high arsenic concentration found in S2 (11100 $mg \cdot kg^{-1}$), similar to the previous sediment, is due to the fact that this point is located just close to the spoil heap. Rainwater percolates the minerals of tailings and spoil heaps, mobilizing and transporting them downstream and hence causing the accumulation of arsenic in sediments in these areas. The lower arsenic concentration in S3 (4770 $mg \cdot kg^{-1}$) indicates the diminution of arsenic content with the distance to the spoil heap. Nevertheless, this value still remains being too high.

The diminution of mercury contents in points S2 and S3 respect to S1 is very remarkable and much greater than for arsenic. Whereas the reduction of arsenic concentration from S1 to S3 is about 56%, for mercury, the decrease represents 91%. It suggests that dispersion of mercury is much localized around the source. This tendency has been observed in previous studies. Fernández-Martinez et al. (2005) [49] reported mercury concentrations of about 100 times lower at a distance of 25 m from the pollution source in a nearby cinnabar area. However, arsenic dispersion is markedly superior, which implies that this element poses higher tendency to be mobilized in this area. Hence, evaluation of the availability of this element and the environmental hazard that it represents for the mine surroundings is necessary.

In order to understand the extent of mercury and arsenic contamination and spread in this site, it is interesting the comparison of the above cited values with the mercury and arsenic background levels in this area. In this sense, a previous sampling survey was undertaken in a neighbouring zone not affected by the mining activity, but with the same geologic and pedological substrate. Nine samples were subjected to the

same methodology of sampling, preparation and analysis that those described in this paper. Background concentrations of mercury and arsenic were 4.18 and 39 $mg \cdot kg^{-1}$, respectively. According to literature, mercury average concentration for uncontaminated soils has been reported about 0.03–0.37 $mg \cdot kg^{-1}$, whereas for areas near mineral deposits it was 0.1–54 $mg \cdot kg^{-1}$ [62]. The average arsenic concentration in uncontaminated soils is 5–10 $mg \cdot kg^{-1}$ [1], but for areas near mineral deposits it may range from 400 to 900 $mg \cdot kg^{-1}$ [24]. Comparatively, the mercury background value found in this area is in the normal range of surrounding areas of mines, whereas for arsenic the local background is notably lower than those reported for areas near mines. Nevertheless, the arsenic background value is very close to the toxicity threshold value accepted for arsenic (40 $mg \cdot kg^{-1}$) [63]. When compared the arsenic and mercury concentrations in S3 to the values of uncontaminated soils, it is deduced that this point is notably more polluted by arsenic.

4.3.3.1.2 *La Peña-El Terronal*

Results from the physicochemical characterization were globally similar to those observed for La Soterraña. However, arsenic concentration in point S6, 350 $mg \cdot kg^{-1}$, is markedly lower than in sediment samples from the previous mine, and lower than the reported values for areas near mining deposits [24]. Hence, the encapsulation carried out in the main spoil heap of this mine is effective in reducing arsenic levels.

The geochemical background for the area near the mine not affected by mining works is 3-4 $mg \cdot kg^{-1}$ for mercury and 6-20 $mg \cdot kg^{-1}$ for arsenic [51, 64] and the mercury concentration corresponding to S6 is 1.87 $mg \cdot kg^{-1}$, even lower than the background value, which reinforces the fact of the effectiveness of the spoil heap encapsulation.

4.3.3.1.3 *Los Rueldos*

The situation in this mine is completely different to the previous ones. The acid pHs and high Ehs in local sediments indicate that AMD occurs in this mine site. In addition, the high sulphur and iron contents in points S8 and S9 are indicative of the notably presence of pyrite and other sulphides in the area. It is noteworthy the very low calcium contents in these samples, suggesting the non-calcareous nature of soils and rocks at this mine site. Likewise, manganese contents are lower than those found in La Soterraña or La Peña sediments. Arsenic content in S8 is extremely high (30700 $mg \cdot kg^{-1}$). The reason is the location of this sediment, situated in a small pond, just at the entrance of a mine gallery, with a very low water flow that receives and

accumulates the very fine-grained sediments carried by the mine water along. However, arsenic and mercury contents are lower in S9 (11786 $mg \cdot kg^{-1}$), since this sediment is located inside the gallery, and corresponds to the fine material washed by mine water flowing inside.

The local geochemical backgrounds in soils of this area are 1.6 $mg \cdot kg^{-1}$ for mercury and 8 $mg \cdot kg^{-1}$ for arsenic [52]. They are markedly lower than those reported for La Soterraña, or other mining areas, as in The Ervedosa mine (Northeaster Portugal) where arsenic background is 218 $mg \cdot kg^{-1}$ [65]. This entails that the arsenic and mercury concentrations found in sediments of this mine are caused by anthropogenic activities, rather than for the mineralogy of the area.

4.3.3.1.4 *Santa Águeda*

Similarly to La Soterraña and La Peña, samples collected at this mine present neutral pHs and moderately oxidant Ehs.

The spoil heap sample R1 presents the highest arsenic contents of all the studied samples (67000 $mg \cdot kg^{-1}$), value comparable to those found in tailings from Daduck mine in Corea (up to 56600 $mg \cdot kg^{-1}$) [66] and in slag fragments from an industrial site dedicated to ore processing in Belgium [67] (67200 $mg \cdot kg^{-1}$ as average content). In literature reviews, up to several hundreds of $mg \cdot kg^{-1}$ have been reported for mine tailings [68, 69]. The observed extremely elevated arsenic concentration is not surprising since this sample corresponds to an arsenic mine spoil heap. However, arsenic concentrations in R5 and R6 are not so high (≤ 1000 $mg \cdot kg^{-1}$). It is also very striking the large calcium an iron contents in R1. As regards mercury, its presence in this points is not as important as in the previous mine sites, due to the nature of the ores and the mineral paragenesis of the deposit.

Local geochemical background concentrations in soils of the area are 150 $mg \cdot kg^{-1}$ for arsenic and 0.6 $mg \cdot kg^{-1}$ for mercury [43]. The arsenic background value is the highest among all the studied mine sites, due to the geological As-enrichment of sulphide ores in this area.

4.3.3.2 Partitioning of arsenic and related elements in the studied mines

The arsenic fractionation results in the studied samples from each mine site are summarized in Fig. 4.3.2 and the distribution of the selected major elements is exposed in Fig. 4.3.3.

Recovery of arsenic, calculated as the sum of arsenic concentrations leached in every step compared to total arsenic content, range from 79% to 98% for all the samples.

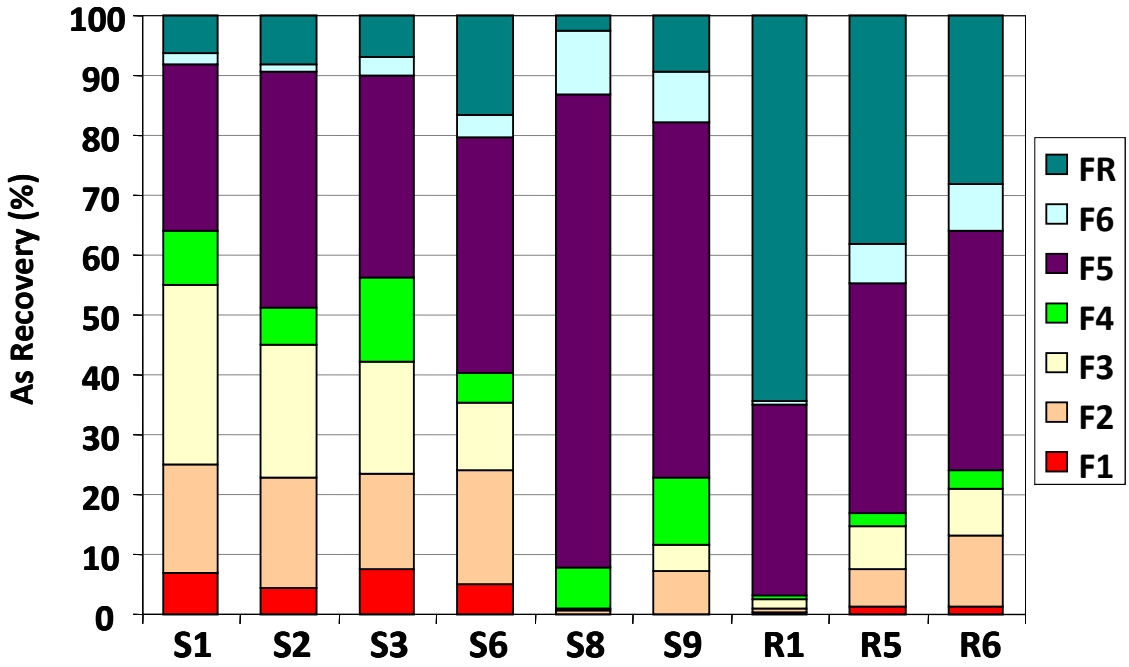


Fig 4.3.2. Arsenic partitioning in all the studied samples.

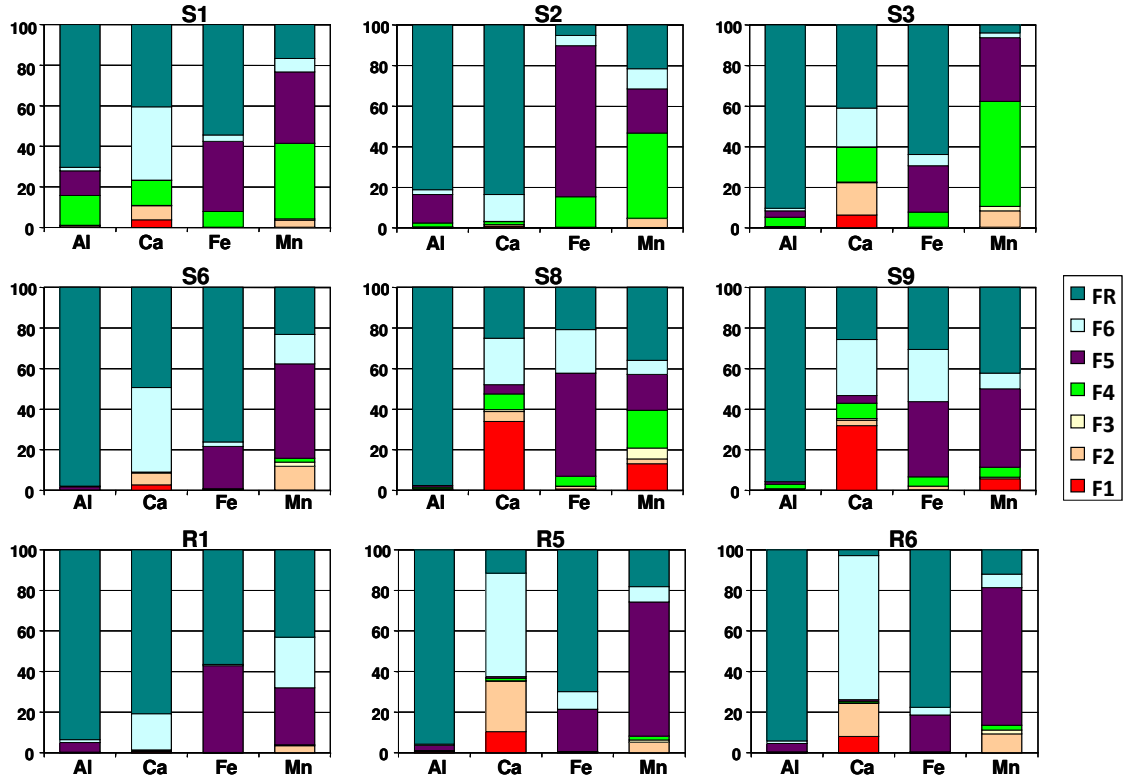


Fig 4.3.3. Partitioning of some major elements in all the studied samples. In y-axis is represented arsenic recovery (%).

4.3.3.2.1 *La Soterraña*

Arsenic distribution is very similar in the three studied samples from La Soterraña. S1, S2 and S3 present the highest percentages of arsenic leached in fractions F1 (between 4.5 and 7.6%) and F2 (between 15.8 and 18.3%), exhibiting the largest arsenic mobility of all the studied sediments, since between 23 and 25% of arsenic (sum of F1+F2 arsenic contents) can be considered as bioavailable [70].

The percentage of arsenic soluble in water is notably higher than those found in the literature. A content of 1% of arsenic water-extractable was reported for the certified reference material Montana soil (SRM2710) [9]; a mean value of 0.18% of arsenic was reported for mining soils from Salamanca [71] and similar contents in other polluted mining soils [72, 73]. The appreciable amounts of calcium in these sediments can involve the existence of calcium arsenates, since it is considered that the bulk of secondary minerals are composed of arsenates [4]. Taking into account the moderated solubility of calcium arsenates in water at pH close to neutrality [74], the arsenic extracted in F1 can be a consequence of the solubilisation of these species.

The percentages of arsenic extracted in F2 are higher than 10% given by Wenzel et al. (2001) [75] for soils contaminated by arsenic in Austria, but much lower than the above 50% reported for wetland and river sediments [76]. The adsorption of arsenic onto calcium carbonates may occur given the important presence of calcite in the samples [77], Calcium carbonate minerals, especially calcite, can provide sites for arsenic retention [12, 78-82]. Arsenate can interact specifically with surface structural Ca^{2+}, probably as outer sphere complexes [15, 83]. Arsenic bound to carbonates is supposed to be released with Na_2HPO_4 in F2 by competitive desorption with phosphate anions. This can explain the fact that F2 has more importance for sediments from La Soterraña. This assumption is supported by the important correlation found between arsenic and calcium fractionations, especially in sample S1 (R= 0.7361) and in S3 (R= 0.6088) (Table 4.3.3).

The association of arsenic with organic matter can not be overlooked for any of the three sediments, since up to 14% of arsenic (corresponding to S3) is leached in F4. For tailings from Myoungbong mining, in South Corea, the arsenic bound to soil organic matter accounted for 23% [66].

Nevertheless, in these samples the bulk of arsenic is associated with oxyhydroxides (F3+F5+F6). For S1, the highest proportion of arsenic can be supposed

linked to Al oxyhydroxides (F3). Cappuyns et al. (2002) [67] found that in samples polluted during industrial production of metal alloys and arsenic compounds the larger part of arsenic, up to 25%, was released during NH_4F extraction. However, in samples S2 and S3 arsenic is mainly associated with Fe oxyhydroxides (mainly F5). This is consistent with the fact that mine wastes from this area are decomposed in oxidant conditions and sulphides are usually in advanced states of oxidation, with Fe oxyhydroxides as coatings. It is striking that, whereas arsenic incorporated into amorphous Fe oxyhydroxides (28-39%) was the predominating fraction (F5), arsenic associated with poorly crystalline Fe oxyhydroxides (F6) remained <3% for the three samples. This proportion is according to iron fractionation profile (Fig. 4.3.3) where an important part of iron is leached during F5 step. In the case of sample S2 arsenic mobility is more similar to iron mobility (R= 0.7603) (Table 4.3.3), which seems logical since this sediment sample presented the higher association with iron phases among the three samples from La Soterraña.

The low arsenic content found in the residual fraction (<10% of total arsenic) is related to the origin of samples since they were collected in the surroundings of mine spoil heaps where large volumes of roasted rock and mine-ore waste materials are present. These waste spoil heaps are characterized by its red-brown color due to the presence of abundant iron oxides as fine-grained particles. Retorting of Hg-bearing ore is known to be an inefficient and incomplete process, and as a consequence, sulphide minerals can be converted to higher mobile phases as sulphates, arsenates and oxyhydroxides [84, 85]. These values are in agreement with those found in samples from Ervedosa Mine [65].

4.3.3.2.2 *La Peña-El Terronal*

The distribution of arsenic in S6 is rather similar to sediments from La Soterraña. The percentage of bioavailable arsenic represents more than 24% of total arsenic (5% solubilised in water (F1) and 19% extracted with phosphate (F2) which means that, in spite of greatly decreasing total arsenic concentration, encapsulation in concrete is not effective in reducing the mobility of arsenic-rich wastes. As in the previous samples, arsenic is primarily associated with amorphous iron oxyhydroxides (F5), which is in accordance with iron partitioning. An important correlation between arsenic and manganese distributions (R = 0.8806) was found. Mn-bearing phases have been described in literature as important mineralogical phases for arsenic sorption and coprecipitation [5]. In addition, manganese profile (Fig. 4.3.3) indicates that arsenic association to manganese oxyhydroxides is a predominant process in this area.

Table 4.3.3. Pearson correlation coefficients between fractionation of As and other major elements.

Sample	Al	Ca	Fe	Mn
S1	0.2903	0.7361	0.4196	0.0735
S2	0.0794	0.2814	0.7603	0.0283
S3	0.2960	0.6088	0.0557	0.4665
S6	0.0917	0.1828	0.0949	0.8806
S8	0.1769	0.3205	0.9038	0.1345
S9	0.3114	0.3801	0.6834	0.6305
R1	0.9051	0.8401	0.9757	0.8578
R5	0.6492	0.2482	0.6107	0.8107
R6	0.4605	0.2474	0.8181	0.8740

4.3.3.2.3 *Los Rueldos*

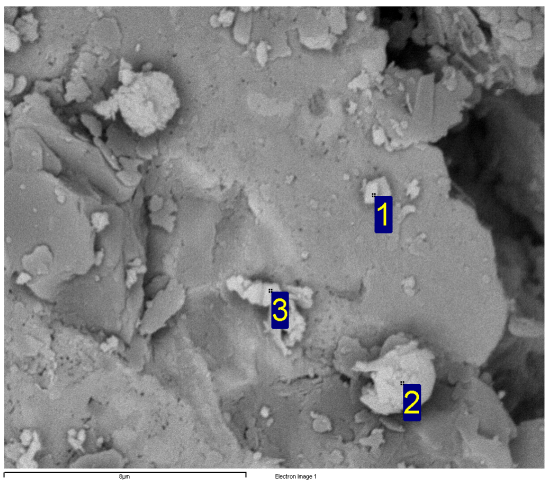
The preferences of arsenic associations with solid phases in samples from Los Rueldos (S8 and S9) differ greatly from the before studied samples. In fact, negligible amounts of water-soluble arsenic (F1) were observed, comparable to those for samples from Everdosa Mine [65], as well as the strongly adsorbed arsenic fraction (F2) was significant only in sample S9 (7% of total arsenic) and negligible for sample S8. From these results, it is evident that arsenic bioavailability in these sediments is notably lower than for samples from La Soterraña and La Peña.

Certain association of arsenic with organic matter also occurs in both sediments (6.7 and 11.4% extracted in F4 for S8 and S9 respectively). Huang and Kretzschmar (2010) [86] found that this fraction was very important in all soils, regardless of their nature. Arsenic it has been hypothesized that arsenic association with humic acid occurs through bridging metals [28], the high aluminium and iron concentrations in S8 and S9 may explain the significant presence of arsenic concentration in fraction F4.

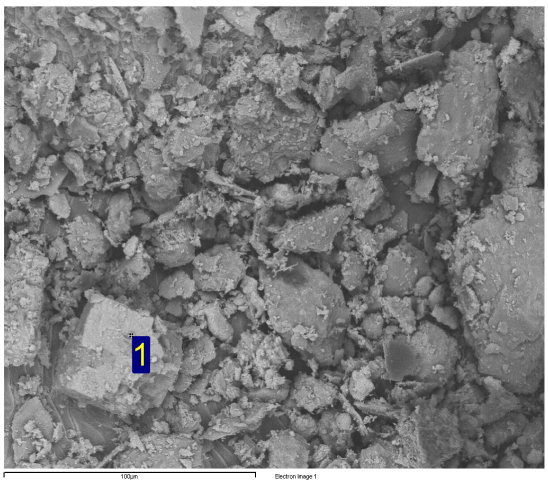
Arsenic was mostly bound to iron oxyhydroxides in both samples, accounting the extracted arsenic in (F5+F6) for more than 90% for S8 and 68% for S9. This association was also confirmed by SEM-EDAX analyses (Fig. 4.3.4). Arsenic contents as high as 14% were detected on individualized particles of amorphous and crystalline iron oxides (goethite). However, the higher association occurs with the amorphous forms (80 and 59% respectively for S8 and S9 in F5). Similar values for this association has been also pointed out by several authors [4, 75, 87, 88]. Sediments from Los Rueldos presented the highest percentage of arsenic associated to Fe oxyhydroxides in comparison with the other studied mines. The reason may be in the large amounts of iron contents in S8 and S9 and hence the presence of more quantity of Fe

oxyhydroxides in Los Rueldos. This is in accordance with the findings of important amounts of iron oxyhydroxides existing in the corresponding spoil heaps as a result of the extent of sulphide oxidation [52]. Additionally, arsenic is associated to poorly crystalline forms of Fe oxyhydroxides (F6) in greater extent in samples from this mining area. This pattern is in accordance with iron fractionation (Fig. 4.3.3) and the strong correlation found between arsenic and iron (Table 4.3.3).

Again the presence of arsenic forming part of structural minerals results surprisingly scanty (<10%), as in the previous studied mines. Matera et al. (2003) [4] also found that both in soils contaminated by pollution or because of mining, the arsenic that remained in the residual phase was lower than 10%. It means that probably most of arsenic of these samples is the consequence of the conversion and dispersion of primary minerals into other more labile forms because of mining activities and associated weathering.



Spectrum	O	Al	Si	Ti	Fe	As
3	32.90	3.41	6..99	3.60	46.01	7.08



Spectrum	O	Al	Si	Ti	Fe	As
3	46.40	4.69	10.60	1.63	22.41	13.95

Fig. 4.3.4. SEM-EDAX analyses of samples from Los Rueldos.

4.3.3.2.4 *Santa Águeda*

Arsenic distribution pattern in samples from this mining area shows that the bulk of arsenic is mainly bound to amorphous iron oxyhydroxides (F5) and refractory minerals (FR). In fact, for R1, practically the whole of arsenic is just distributed among F5 and FR (32 and 64% of total arsenic, respectively). This is in agreement with results of tailing samples from Daduck mine in South Corea [66], where arsenic was also essentially forming part of oxides and refractory minerals. These great arsenic

percentages in residual and oxalate fractions are expected, since these samples correspond to an arsenic mine where mine wastes are primarily constituted by arsenic-iron sulphide minerals in a low to moderate degree of alteration [43]. The preferential association of arsenic with the residual fraction in sample R1 is corroborated with the strong correlation found among arsenic and all the studied major elements (Table 4.3.3). It means that in the upper waste pile materials remain mostly as unaltered (or low altered) primary minerals. However, in R5 and R6, although the percentage of arsenic extracted in F5 is similar to R1, the amount of arsenic coprecipitated with structural minerals is lower (38% in R5 and 28% in R6), which can be explained by the fact that these sampling points are located downstream of the mine and receive the runoff from the upper mine wastes. In consequence, they exhibit higher arsenic mobility and other fractions are quantitatively important, as arsenic adsorbed onto mineral surfaces (F2) and the association of arsenic with Al oxyhydroxides (F3). The extracted arsenic percentage in F2 is in the same level than those reported in soils of different nature [86], and the NH_4F-extracted arsenic comparable to the percentage leached in the certified reference materials Montana soil (SRM2710) and the stream sediment (GBW07311) [9].

In contrast to sample R1, significant arsenic concentrations associated to weakly crystalline Fe oxyhydroxides appear in R5 and R6. This is in agreement to iron distribution (Fig. 4.3.3) which demonstrates the occurrence of crystalline iron oxyhydroxides in samples R5 and R6.

The mobility of arsenic in sample R5 is more related to manganese (R= 0.8107), and in sample R6 with iron and manganese (R= 0.8181 and 0.8740 respectively) (Table 4.3.3).

These results indicate that arsenic is more available in R5 and R6 than in R1. Nonetheless, the high total arsenic concentration in R1 suggests that environmental concern should be also taken into account for this sample.

4.3.4 CONCLUSIONS

Arsenic partitioning obtained from the application of the proposed sequential extraction protocol to samples from different mining areas was consistent with the sources of arsenic pollution, the mineralogy of the wastes and the extent of mining

activity. These results demonstrate the suitability of the proposed method to evaluate arsenic mobility in a variety of environmental solid samples.

The spread of arsenic pollution is very remarkable in three of the four studied mines, as the high arsenic total contents show, up to 1.1%, 3.1% and 6.7% in La Soterraña, Los Rueldos and Santa Águeda respectively. The exception is La Peña-El Terronal, where the encapsulation carried out in the main spoil heap has as consequence the notably diminution of arsenic contents in soils and sediments.

Arsenic distribution is highly influenced by the extent of mining and ore processing. It seems to be a general rule that the most relevant association of arsenic occurs with iron oxyhydroxides. This fact is more noticeable for samples from Los Rueldos, where up to 90% of total arsenic has been found associated with these species. The highest arsenic mobility was found in samples from La Soterraña and La Peña-El Terronal mines and is related to the great amount of wastes generated by roasting of ore. In these areas more than 20% can be considered as immediately bioavailable. On the contrary, higher association of arsenic with refractory materials as sulphide minerals was found in samples from Santa Águeda mine because of the non-existence of metallurgical treatment of the exploited ore at this site.

Finally, the extremely high arsenic concentrations and its relatively mobility are of environmental concern, since they represent a potential source of arsenic pollution with an associated risk. With the aim of immobilizing arsenic, remediation measures and encapsulation of spoil heaps are highly recommended.

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5. ARSENIC IN MINE WATERS

The presence of arsenic in waters from the surroundings of the studied mine sites may entail an evident risk to those ecosystems, since this contamination may reach rivers and streams flowing through those areas, causing dispersion of this pollutant.

This chapter is an evaluation of the extent of arsenic pollution in surrounding waters of three mercury mines, mainly provoked by the solubilisation of this pollutant from soils, sediments and waste piles of those areas as a result of weathering processes or changes in the environmental conditions of the cited areas.

For assessing the factors controlling arsenic release in every mining area an extensive study based on the physicochemical characterization and multivariate statistical analysis of waters upstream and downstream of each mine site will be performed.

As the toxicity of arsenic depends on its chemical form, determination of arsenic speciation in waters is important for risk analysis assessment. The hyphenated techniques HPLC-HG-AFS will be selected for carrying out the speciation analysis.

In order to assure speciation results, the preservation of the original arsenic speciation during the sample storage until the subsequent analysis is a key question. Hence, different preservation procedures will be tested with the aim of achieving an efficient way of sample stabilization over time.

Possible seasonal variations of arsenic concentrations in the studied waters will be evaluated along a whole year. The dependency of the most important factors controlling arsenic speciation and mobilization (pH and Eh) on the rainfall regime will be also studied.

The information provided by all these studies constitutes a complete evaluation of the impact of arsenic pollution in waters from the studied areas and a comprehensive understanding of the real hazard that this metalloid may cause to their surroundings.

5.1 Arsenic contamination and speciation in surrounding waters of three old cinnabar mines

ABSTRACT

The impact of arsenic pollution in waters from the surroundings of three abandoned Hg mines in Northern Spain, as well as reaching Caudal River, was evaluated. For assessing the factors controlling arsenic release an extensive study based on the physicochemical characterization and multivariate statistical analysis of waters upstream and downstream each mine site was performed. Waters downstream La Soterraña mine present the highest arsenic concentrations, up to 38.8 $mg \cdot l^{-1}$, coming mainly from the solubilisation of calcium, magnesium and strontium arsenates at pH close to neutrality. Although arsenic concentrations downstream La Peña are markedly lower, these values remain being too high, indicating that the encapsulation carried out in this spoil heap is insufficient. In addition, the high water flow in this point involves an extremely high input of arsenic to the surroundings (0.3 $g \cdot s^{-1}$). Waters close to tailings from Los Rueldos suffer from AMD, provoking an important solubilisation of arsenic and heavy metals, situation rapidly softened with distance.

The study of arsenic speciation reveals the omnipresence of As(V) in waters from the three mines, whereas in La Peña low amounts of As(III) were also detected. Different preservation ways for arsenic speciation were compared, as the addition of HCl, EDTA and the storage of samples without any additive, observing no alteration of samples in any case up to nine months before the collection.

A study of seasonal variations of arsenic and the main parameters affecting its concentration and speciation was completed along a year, showing no remarkable dependency with rainfall for any studied variable.

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5.1.1 INTRODUCTION

Arsenic (As) is an element naturally present in the environment. Long before man's activities had any effect on the balance of nature, arsenic was distributed ubiquitously throughout earth crusts, soil, sediments, water, air and living organisms [1]. The major occurrence of arsenic is due to the mineralogical and geological characteristics of the areas and most of environmental arsenic problems are the result of mobilization under natural conditions. However, anthropogenic activities such as mining works and ore processing, combustion of fossil fuels, the use of arsenical pesticides, herbicides and other uses of arsenic have involved an important additional impact [2, 3].

Arsenic is recognized as one of the most serious inorganic contaminants in drinking water on a worldwide basis. Its toxicity depends on the chemical species it presents [4]. It has been well established that organic arsenic compounds are less toxic than the inorganic forms, and among them, As(III) is considered 10 times more toxic than As(V), due to its higher mobility [5-8]. In natural waters, arsenic is mostly found as inorganic forms, although certain amounts of monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) can be present as a result of microbial activity [9]. Inorganic arsenic species dominate in sulphide oxidation environments [10]. Water is the main way of exposure of arsenic to humans, so regulation about maximum allowed values is needed. Whereas the WHO guideline [11] value for arsenic in drinking water is 10 $\mu g \cdot l^{-1}$, the Spanish legislation limits total arsenic content to 500 $\mu g \cdot l^{-1}$ for mining and industrial effluents and 50 $\mu g \cdot l^{-1}$ for waters for production and drinking water.

Arsenic mobility is directly related to arsenic speciation, and it is highly dependent on the physicochemical conditions of the site. It has been widely studied that redox potential (Eh) and pH are the most important factors controlling arsenic speciation [9, 12]. The hazardous potential of arsenic relies on its relative mobility over a wide range of redox conditions, which make arsenic different and unique among the metalloids and oxyanion forming elements. In general, the occurrence of the different forms of arsenic depends on the aerobic and anerobic conditions: arsenite [As(III)] is normally the predominant species in groundwater at anaerobic conditions, whereas arsenate [As(V)] is mainly present in surface waters assuming aerobic conditions. It is well known that arsenate is usually adsorbed on mineral phases in greater extent than arsenite, and this adsorption highly depends on pH values. Roussel et al. (2000) [13] and Daus et al. (1998) [14] postulated that arsenic speciation is controlled by the pH-affected speciation and solubility of iron. In acid mine drainage (AMD) with pH lower

than 3.5, the Eh–pH relation shows that Fe(II) is strongly susceptible to oxidation to Fe(III), which then precipitates as oxyhydroxide or oxyhydroxysulphate. In oxidizing environments, the main attenuation mechanism of arsenic migration is its adsorption on Fe(III)-precipitates, however, As(V) is less soluble and it is adsorbed more efficiently than As(III) under acidic conditions [15]. Hence, in acid mine waters dissolved arsenic is quickly removed as the iron is oxidized and precipitated and the arsenic scavenged through adsorption [9].

Abandoned mines have left behind toxic pits and acid mine drainage. Naturally occurring minerals were disturbed, crushed during ore treatment and left in massive tailing piles. As a consequence potentially toxic materials containing arsenic and heavy metals can be found in dangerously high levels throughout historic mining regions. Natural weathering can lead the mobilization of arsenic to the environment and the solubilisation into the surrounding waters. As usually occurs as As-bearing sulphides, predominantly pyrite. During periods of excess oxygenation pyrite is dissolved allowing arsenic to enter the aquifers [16, 17]. Oxidation of pyrite ore is a major cause of AMD [16, 18, 19]. The production of Fe(III) and hydrogen ions can catalytically dissolve secondary minerals, thereby increasing the metal load to the drainage [19]. In addition, the generally low pH (~1–4) of AMD systems also contribute to the solubilisation of heavy metals [20].

Therefore, although at a world scale typical concentrations for arsenic in non-polluted stream waters have been estimated in 4 $\mu g \cdot l^{-1}$ [21], in the case of waters affected by mining activities these values can increase greatly. Arsenic concentrations up to 556 $\mu g \cdot l^{-1}$ were found in streams adjacent to tailings deposits in British Columbia [22]. Williams et al. (1993) [23] and Smedley et al. (1996) [24] noted around 200–300 $\mu g \cdot l^{-1}$ of arsenic in surface waters affected respectively by Sn in the Nakhon Si Thammarat Province [23] and because of Au mining activities in the area of Ghana [24]. In waters affected by AMD extreme arsenic concentrations have been reported. Plumlee et al. (1999) [25] found up to 340000 $\mu g \cdot l^{-1}$ in Various, USA [25]. Sarmiento et al. (2009) [26] reported arsenic concentrations of 1975 $\mu g \cdot l^{-1}$ and 441 $\mu g \cdot l^{-1}$ for Tinto river and Odiel river respectively in Spain [26]. Higher arsenic concentrations, up to 850000 $\mu g \cdot l^{-1}$ were found by Nordstrom and Alpers (1999) [27] in surrounding waters of The Richmond Mine of the Iron Mountain copper deposit [27].

Asturias in northwestern Spain, is a historical mercury mining district, where mining activity was developed in different periods from the Roman period (first and second centuries) until the seventies in the twenty century when the mining activity was

definitively abandoned. Deposits are located in Precambrian to Carboniferous formations and although the most frequent hosted rocks are limestone, some of the richest deposits are located in conglomeratic horizons or siliceous breccias in sandstones. Ore deposits appear as irregular veins, granular aggregates or impregnations in rocks, commonly associated to fractured rocks with high porosity. The paragenesis of cinnabar ore includes the presence of arsenic sulphide minerals, mainly arsenian pyrite, realgar and orpiment. From a hydrogeological point of view, the substrate of the mine areas is mainly constituted by alternation of limestone, sandstone and shale. Whereas the shale can be considered impermeable, limestone and sandstone are permeable formations and constitute small aquifers. Mines have been exploited by underground mining, usually by the chamber and pillars method, at La Peña-El Terronal site, the underground mine works reach more than 500 meters depth. Smelting operations were accomplished at the most important mine sites for decades, being the ore processed on site in large rotary furnaces. More than 20 abandoned mercury mine sites are scattered throughout the region, but the most important ones are located in central Asturias, in the Caudal River basin. Because of the great decline on Hg prices on the 1970s, most mines were closed between 1973 and 1974, but sometimes any preventive measure was carried out after the closures. Thus mine facilities and metallurgical installations remain currently abandoned, as well as their corresponding spoil heaps. It poses an evident risk, since natural weathering or variations in physicochemical parameters at the disposal site can alter the stability of waste materials, including the As-rich ores, and lead to the release of marcury, arsenic and associated heavy metals into underlying aquifers and superficial waters, which eventually discharge to streams and rivers. Studies about the environmental impact of Asturian abandoned Hg mines are abundant [28-34] and prove the existence of pollution in soils, surface waters, sediments and even in plants of the surroundings [35, 36]. However, no studies exist about the arsenic species occurring in surrounding waters of these mining areas.

Hence, the objectives of this work are: i) evaluation of the impact of arsenic pollution and assessment of factors controlling arsenic release based on physicochemical characterization and statistical studies of waters upstream and downstream of each mine site; ii) study of the arsenic speciation in waters from three abandoned Hg mining areas; iii) study of alteration of arsenic species with different preservation procedures just after the sampling collection and over time; iv) investigation of seasonal variations in arsenic contents and other associated parameters.

5.1.2 EXPERIMENTAL

5.1.2.1 Studied area description and sampling points

The studied areas correspond to three Hg mine sites of Asturias: La Soterraña, located in the vicinity of the village of Pola de Lena, and La Peña-El Terronal and Los Rueldos, located close to Mieres. As it is shown in Fig. 5.1.1, runoff from spoil heaps of the three mines discharge in the Caudal River catchment. The Caudal River is a tributary of Nalón River, the main river of the region.

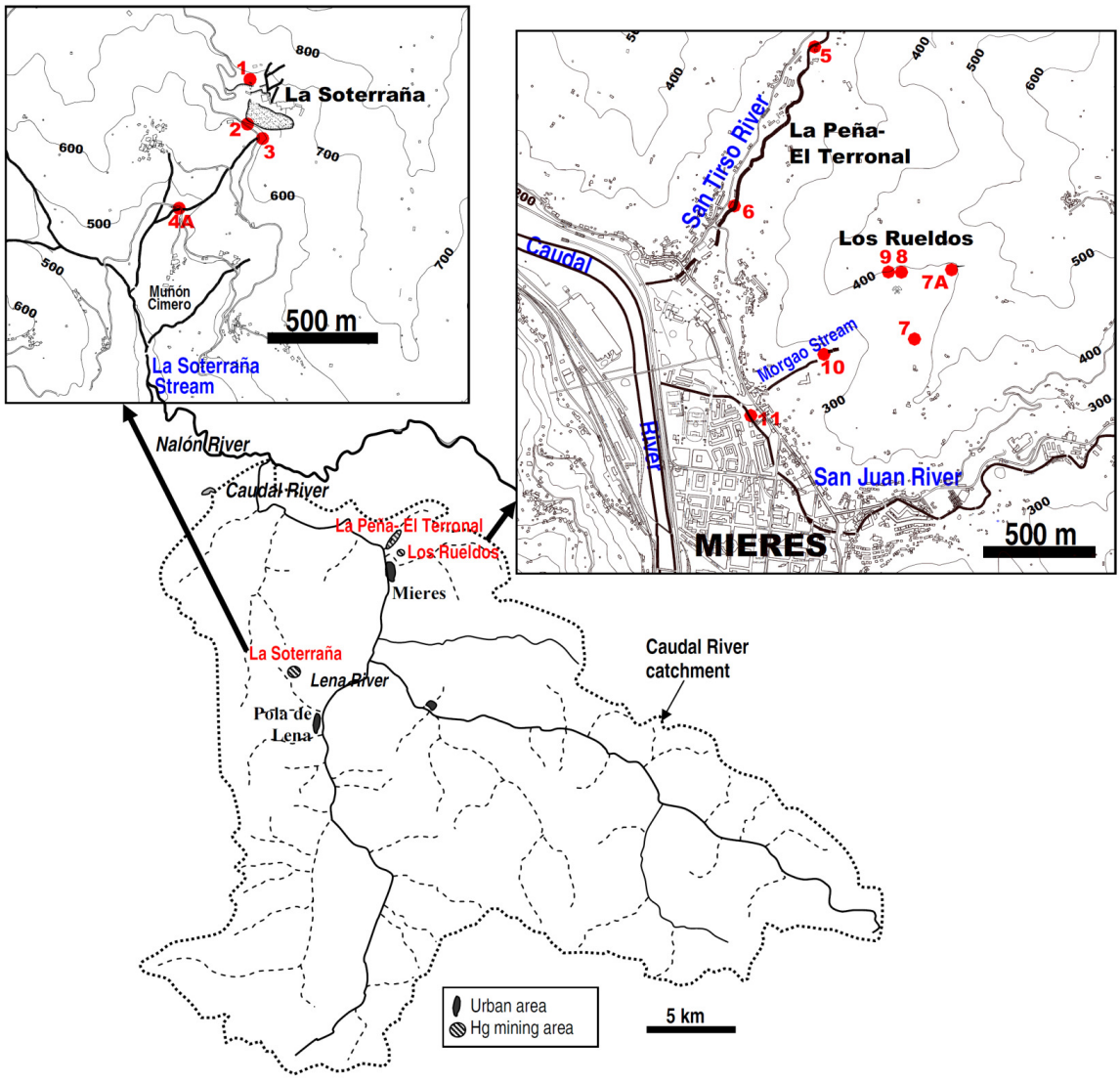

Fig. 5.1.1. Map with the three mines and sampling points.

The presence of arsenic is very frequent in the form of orpiment, realgar and As-rich pyrite [37, 38]. Other primary metallic minerals which are present in the paragenesis of the ore deposits are: pyrite, melnikovite, sphalerite, marcasite,

chalcopyrite, arsenopyrite, galena, and stibnite. Smithsonite, hemimorphite, cerusite, goethite, malachite, jarosite, melanterite and gypsum are present as secondary minerals. From an environmental point of view, the presence of arsenic minerals in the ore deposit paragenesis is specially significant, as high arsenic concentrations have been found in pyrites and iron oxides [31].

In contrast to other regions of Spain, Asturias has a humid and temperate climate characterized by abundant rainfall during a great part of the year. The annual average maximum and minimum temperatures of the last twenty years are 17 and 8 ºC respectively, and the annual average relative humidity ranges from 74 to 84%. Average yearly rainfall in the same period was 966 mm and annual potential evapotranspiration calculated by Thorntwaite expression is 691 $mm \cdot year^{-1}$. In consequence, the average annual effective precipitation is 275 $mm \cdot year^{-1}$. The one hundred-year 1 day rainfall is 97.8 $mm \cdot day^{-1}$ [32]. These climatic conditions contribute to the spread of the contaminants throughout the area [39].

Sampling points are showed in Fig. 5.1.1 and described in Table 5.1.1. When possible, surface water samples were collected upstream and downstream the spoil heap of each mine.

Table 5.1.1. Description of sampling points.

Mine site	Sampling point	Description
La Soterreña	P1	Surface runoff (upstream of La Soterraña Mine)
	P2	Air-open channel (downstream of La Soterraña Mine, receiving spoil heap leachates)
	P3	Brook at the bottom of La Soterraña spoil heap
	P4A	Stream (downstream of La Soterraña mine site receiving spoil heap leachates)
La Peña-El Terronal	P5	San Tirso River (upstream of La Peña-El Terronal mine site)
	P6	San Tirso River (downstream of La Peña-El Terronal mine site)
Los Rueldos	P7A	Spring upstream of Los Rueldos mine site
	P7	Surface runoff upstream of Los Rueldos mine site
	P8	Los Rueldos mine drainage (water pond at the entrance of the gallery)
	P9	Los Rueldos mine drainage (water inside the gallery)
	P10	Morgao stream (downstream of Los Rueldos mine site)
	P11	San Juan River (downstream of Los Rueldos mine site)

La Soterraña mine is located in the northern slope of a deep valley crossed by a small stream, sometimes dry, but whose flow can reach other times up to 0.125 $l \cdot s^{-1}$. This stream collects surface runoff, spoil heap leachates and effluents from a local industry devoted to the manufacture of ceramics, increasing its flow to an average value of 0.166 $l \cdot s^{-1}$ or 0.750 $l \cdot s^{-1}$ as maximum. This stream enters into Lena River, a tributary of Caudal River. The area occupied by the abandoned mining and metallurgical installations, including the spoil heap, is estimated at 72,000 m^2. The substrate of the mineralized area is mainly composed of materials of the "Llanón-Tendeyón" unit, constituted by an alternation of limestones, sandstones and lutites (dominant), which can be considered impermeable excepting some limestones and sandstones bars which constitute small aquifers [31]. Taking into account the climate data and considering an infiltration coefficient of 0.7 for the spoil heap, infiltrated rainwater with possibility to enter in contact with mine waste materials is about 11,781 $m^3 \cdot year^{-1}$. This water transports pollutants in solution to groundwater and/or to the surface watercourses [32].

La Peña-El Terronal mine is located in the valley of San Tirso River, tributary of Caudal River, and whose flow ranged from 76 to 310 $l \cdot s^{-1}$ during the monitored period. The mineralization is included in the Westphalian stratigraphic level which consists of a thick series of shales with intercalations of sandstones, conglomerates, limestones and coal beds usually less than 50 cm thick. The carboniferous sediments are overlain discordantly by Upper Stephanian – Lower Permian materials of the San Tirso formation, comprising calcareous conglomerates, clay lutites, and tuffaceous materials [40]. Contrary to the other two mines, where any restoration program was implemented after the closure of the mines, a remediation plan consisting of the encapsulation of the main spoil heap was carried out in this mine in 2002, when 300,000 tons of spoil heap wastes (constituted by tailings and granulated materials of various sizes) were isolated in an on-site security landfill [39].

Los Rueldos mine is crossed by Morgao stream, being canalized underground, downstream of the mine site. Mine drainage and spoil heap leachates reach Morgao stream when it reappears on surface. Low water flow rates are registered upstream where even is absent during summer months. At the end of the canalization (P10), water flow ranges from 0.075 to 2.1 $l \cdot s^{-1}$, whereas in P11 (San Tirso River), it remains very low [32]. From a hydrogeological point of view, the substrate of the catchment (alternance of sandstones, shales and some coal beds) can be considered as an impermeable substrate. In these conditions, the water does not evaporate nor flows superficially; but it is infiltrated on the more permeable colluvial materials and

weathered shales, giving rise to a number of small springs. In this case, downstream of the Hg mining works, there is a spoil heap constituted by low-permeability wastes from coal washery, and they behave as a pseudokarstic aquifer with capacity to store a great volume of water [34].

5.1.2.2 Water characterization

Water samples were collected, filtered on-site and stored in plastic bottles. Some physicochemical parameters such as pH, specific conductance, dissolved oxygen, salinity, temperature, redox potential and turbidity were measured in the field by means of a portable multiparameter probe (Turo-graph 611). Water flow was measured when it was possible with a current meter (Global Water flow probe). Then, samples were refrigerated at 4 ºC until analysis.

Total arsenic contents, as well as concentrations of major and minor elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a VARIAN 735-ES instrument with radial configuration following the EPA/200.7 method [41, 42]. Quantification limits, calculated as ten times the standard deviation of a blank injected 10 times divided by the sensitivity (slope of the calibration curve), were 0.05 $mg \cdot l^{-1}$ for Al, Ca, Co, Cr, Cu, Mg, Pb, and Zn, and 0.03 $mg \cdot l^{-1}$ for As, Cd, Fe, Mn and Ni.

Na and K were determined by flame atomic emission spectrophotometry (FAES) using a Perkin Elmer 2280 following the EPA/273.1 and EPA/258.1 methods, respectively [43]. Detection limits were 0.1 $mg \cdot l^{-1}$ for both elements.

Cl^-, NO_2^-, NO_3^- and SO_4^- were analysed by means of ionic chromatography following the EPA/9056a method [44]. The used instrument was a Dionex Serie DX-500, equipped with a suppressor device (Dionex CSRS-ULTRA 4 mm). The column in charge of the separation of the anions was a Dionex IonPac AS14 (4 x 250 mm), and its corresponding guard column, Dionex IonPac AG14 (4 x 50 mm). The mobile phase consisted on two solutions: 3.5 M Na_2CO_3 and 1 M $NaHCO_3$. Detection limits were 0.510, 0.055, 0.501 and 0.514 $mg \cdot l^{-1}$ for Cl^-, NO_2^-, NO_3^- and $SO_4^=$ respectively.

Carbonate and bicarbonate anions were analyzed by potentiometric titration with 0.02 M HCl by means of the automatic titroprocessor 796 of Metrohm following the EPA/301.1 method [43]. The detection limits for both of them are 12 $mg \cdot l^{-1}$. Total organic carbon was determined as NPOC (Non Purgeable Organic Carbon) with an

analyzer TOC (Shimadzu–VCHS) by means of oxidative combustion and determination with a non-dispersive infrared sensor (NDIR).

Ultrapure water from a Milli-Q system (Millipore Bedford, MA) was used throughout. All glassware and bottles were cleaned in 0.5 M HNO_3 for 2 days and rinsed three times with deionized water before use. All chemicals used were of analytical-reagent grade (Merck, Darmstadt, Germany). Working standard solutions for ICP-AES and FAES were prepared freshly from individual 1000 $mg \cdot l^{-1}$ stock standard solutions by sequential dilution with 0.5 M HNO_3. Stock standard solutions of arsenic species (1000 $mg \cdot l^{-1}$, as As) were prepared from arsenic trioxide (Panreac, Barcelona, Spain), sodium arsenate (Merck, Darmstadt, Germany), sodium monomethylarsinate (MMA) (Carlo Erba, Milan, Italy) and dimethylarsinic acid (DMA) (Sigma, St. Louis, MO) in distilled water. These solutions were stored in the dark at 4 ºC and their stability frequently checked. As(III) standard solution was kept 1 month to prevent eventual transformation into As(V) species. The others solutions can be considered stable for several months. Intermediate standard solutions (As = 10 $mg \cdot l^{-1}$) were prepared daily and used directly for the preparation of appropriate working standard solutions which were tested every day in the quality control of the analytical procedure.

5.1.2.3 Arsenic speciation

Arsenic speciation studies were carried out by coupled high performance liquid chromatography–hydride generation–atomic fluorescence spectrometry HPLC-HG-AFS. The HPLC system consisted of a Varian Prostar ternary solvent delivery module model 230 equipped with a Rheodyne injector model 7725 and a 200 µl loop for sample introduction. The instrument used in the combination of vapour generation and atomic fluorescence spectrometry was a PSA 10.055 Millennium Excalibur from PS Analytical equipped with an arsenic hollow cathode lamp and a Perma pure drying membrane (Perma Pure Products, Farmingdale, NJ, USA) for drying the generated hydride. The chromatographic separation of As(III), DMA, MMA and As(V) was achieved in this order in 7 min using a Hamilton PRP-X100 anion-exchange column (250 x 4.1 mm id, 10 µm) (Fig. 5.1.2). Chromatographic conditions reported by Gómez-Ariza et al. (1998) [45] were chosen as starting point for the optimization of our chromatographic method. 10 mM and 100 mM phosphate solutions (eluents A and B), buffered at pH 6.2, were used as eluents. Both solutions were freshly prepared, as well as degasified and filtered by 0.45 µm before analysis. The gradient programme was performed as follows: from 0 to 2.0 min: 100% A; from 2.1 to 3.1 min: change to 100% B; from 3.1 to 6.0 min: 100% B; from 6.0 to 7.0 min: change to 100% A. The injected

volume of sample (previously filtered by 0.22 µm) was of 200 µl and the flow rate of mobile phase of 1.0 ml·min^{-1}. The separated arsenic species were subjected to hydride generation with HCl (12.5% v/v, 2 ml·min^{-1}) and $NaBH_4$ (1.4% w/v in 0.1 M NaOH, 2 ml·min^{-1}) in a PEEK mixing cross to generate hydrogen and volatile arsines. The gas-liquid mixture was delivered to a gas-liquid separator, and gas compounds were swept from the mixture with a flow of argon (200 ml·min^{-1}). Arsines entering the atomic fluorescence spectrometer were dried in a Perma-Pure mini-dryer using argon as dryer gas at 100 ml·min^{-1} flow rate. Finally, arsines were atomized in a hydrogen flame aligned in the lightpath of a boosted arsenic hollow cathode lamp and fluorescence emission was detected at 193.7 nm. Detection limits, calculated as three times the standard deviation of a blank injected ten times divided by the sensitivity, were 0.1 µg·l^{-1} for As(III) and DMA, and 0.2 µg·l^{-1} for MMA and As(V).

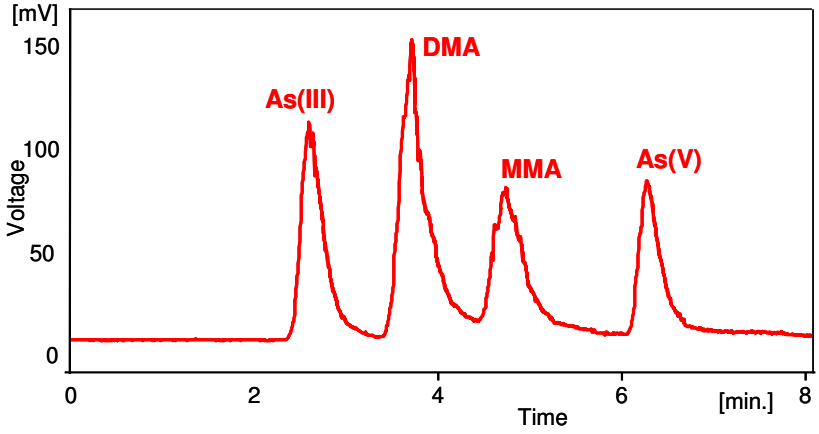

Fig. 5.1.2. Chromatogram of the four arsenic species (100 µg·l^{-1} of each species).

5.1.2.4 Preservation studies for arsenic speciation

Different preservation strategies were tested in order to check the possible alteration of the original speciation of samples with time.

All samples were firstly filtered by 0.45 µm in situ, and then three aliquots were stored in polypropylene bottles of 125 ml. The first aliquot was stored without any stabilization additive; the second one with a few drops of concentrated HCl up to about pH 2; and the third one with a 5% (v/v) of a 0.25 mol·l^{-1} EDTA solution. Finally, all samples were stored at 4 ºC in dark.

The study of As speciation was carried out as soon as it was possible after the sample collection in the field. Time stability of inorganic arsenic species in samples was also checked by speciation studies in all aliquots every three months over one year.

5.1.3 RESULTS AND DISCUSSION

5.1.3.1 Total arsenic contents and associated parameters

Water characterization parameters, as well as total contents of the studied elements for all samples are summarised in Table 5.1.2. Fig. 5.1.3 shows the parameters more influenced by the environmental impact of each mine, and stresses the differences among the three studied mine sites.

Arsenic concentrations in points situated upstream from La Soterraña mine were non detectable. However, the impact of the mine is evidenced by the extremely high levels of arsenic found in waters downstream of the mine operations (up to 38.8 $mg \cdot l^{-1}$ in P3). This clearly indicates that water flowing nearby the spoil heap can mobilize a great amount of arsenic. Data show that these points are not affected by AMD (Acid Mine Drainage), since pH values range about weakly alkaline (7.5 – 8.0) with slightly oxidants values of redox potential (213 – 307 mV). Consequently, dissolution of heavy metals does not occur and conductance (1366 $\mu S \cdot cm^{-1}$ as maximum value after the mine) and salinity (0.68 ppt as maximum after the mine) were not remarkable. The appreciable Ca, Mg and Sr concentrations observed in these samples, especially in points located after the mine (up to 255, 41.7 and 3.09 $mg \cdot l^{-1}$ respectively in P3) suggest the solubilisation of Ca, Mg and Sr arsenates, since it has been reported that at pH ranging between 6.90 and 8.35 Ca, Mg and Sr arsenates are moderately soluble [46, 47]. In addition, the higher sulphate contents downstream compared with those found in P1 indicate that arsenic may be also partially leached because of the oxidation of sulphur minerals. Particularly worrying it is the fact that arsenic concentration does not decrease with the distance to the spoil heap since P3 and P4A have similar arsenic contents. These As-enriched waters are eventually discharged to streams that are incorporated to important rivers at regional level. Arsenic concentrations in these waters (up to 34.4 $mg \cdot l^{-1}$ in P4A, corresponding to a stream) are comparable to those extreme reported by Plumlee et al. (1999) [25] in waters affected by AMD in Various, USA and result clearly higher than those found in other Spanish mining areas affected by AMD. Sarmiento et al. (2009) [26] reported arsenic concentrations of 1.97 $mg \cdot l^{-1}$ and 0.44 $mg \cdot l^{-1}$ for Tinto river and Odiel river respectively. Higher arsenic concentrations up to 850 $mg \cdot l^{-1}$ were found by Nordstrom et al. (1999) [27] in surrounding waters of The Richmond Mine of the Iron Mountain copper deposit.

Table 5.1.2. Water characterization for all tested points in each mine.

Sampling point	La Soterraña				El Terronal	
	P1 (upstream)	P2 (downstream)	P3 (downstream)	P4A (downstream)	P5 (upstream)	P6 (downstream)
Flow ($l \cdot s^{-1}$)	0.077	0.129	0.173	0.196	99	110
S. Conductance ($\mu S \cdot cm^{-1}$)	462	762	1366	1133	1105	886
Salinity (ppt)	0.21	0.36	0.68	0.54	0.54	0.42
Turbidity (NTU)	34.5	75.9	43.4	57.0	38.4	33.4
Temperature (ºC)	13.7	11.1	12.2	11.4	12.5	13.9
pH	7.5	8.0	7.8	8.0	7.8	8.2
Eh (mV)	307	262	259	213	215	204
Dissolved oxygen ($mg \cdot l^{-1}$)	6.55	9.63	7.25	6.84	8.24	6.82
TOC ($mgC\ l^{-1}$)	*	7.40	4.30	4.07	20.3	17.9
Cl^- ($mg \cdot l^{-1}$)	*	13.0	18.4	16.8	13.6	13.2
HCO_3^- ($mg \cdot l^{-1}$)	*	198	171	189	252	224
NO_2^- ($mg \cdot l^{-1}$)	*	n.d.	0.18	n.d.	0.19	0.34
NO_3^- ($mg \cdot l^{-1}$)	*	1.40	0.86	2.62	2.80	3.12
SO_4^{2-} ($mg \cdot l^{-1}$)	*	267	613	405	255	222
Al ($mg \cdot l^{-1}$)	n.d.	n.d	n.d	n.d	n.d	n.d
As ($mg \cdot l^{-1}$)	n.d.	21.6	38.8	34.4	0.12	3.16
Ca ($mg \cdot l^{-1}$)	69.0	112	225	177	103	101
Cd ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Co ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cr ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cu ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fe ($mg \cdot l^{-1}$)	0.05	0.11	0.05	0.04	0.05	0.20
K ($mg \cdot l^{-1}$)	2.44	4.00	12.2	11.1	6.03	5.89
Mg ($mg \cdot l^{-1}$)	14.8	18.6	41.7	29.5	56.6	52.3
Mn ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Na ($mg \cdot l^{-1}$)	4.54	12.6	15.8	16.2	10.7	14.9
Ni ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sr ($mg \cdot l^{-1}$)	0.17	0.66	3.09	1.83	0.34	0.37
Zn ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 5.1.2. (continuation from previous page)

Sampling point	Los Rueldos					
	P7A (upstream)	P7 (upstream)	P8 (downstream)	P9 (downstream)	P10 (downstream)	P11 (downstream)
Flow ($l \cdot s^{-1}$)	0	0.021	0	0	0.643	0.033
S. Conductance ($\mu S \cdot cm^{-1}$)	211	406	5248	5523	1228	1107
Salinity (ppt)	0.98	0.89	39.3	29.7	0.57	0.53
Turbidity (NTU)	47.0	39.4	119	22.8	49.8	51.3
Temperature (ºC)	14.5	18.5	14.0	12.4	13.7	13.2
pH	7.6	8.2	2.2	2.2	8.2	8.0
Eh (mV)	190	169	654	669	382	277
Dissolved oxygen ($mg \cdot l^{-1}$)	7.87	6.95	8.36	7.12	11.7	7.37
TOC ($mgC\ l^{-1}$)	6.85	16.7	5.60	4.73	*	2.53
Cl^- ($mg \cdot l^{-1}$)	10.2	10.1	6.9	7.0	*	14.6
HCO_3^- ($mg \cdot l^{-1}$)	58	166	n.d.	n.d.	*	278
NO_2^- ($mg \cdot l^{-1}$)	n.d.	n.d.	n.d.	n.d.	*	0.14
NO_3^- ($mg \cdot l^{-1}$)	0.82	1.10	0.70	0.23	*	3.20
SO_4^{2-} ($mg \cdot l^{-1}$)	37	55	4200	4500	*	351
Al ($mg \cdot l^{-1}$)	n.d	0.18	150	145	0.87	0.50
As ($mg \cdot l^{-1}$)	n.d.	n.d.	7.2	7.2	1.00	0.13
Ca ($mg \cdot l^{-1}$)	25.3	56.7	134	136	111	105
Cd ($mg \cdot l^{-1}$)	n.d.	n.d.	0.01	0.01	n.d.	n.d.
Co ($mg \cdot l^{-1}$)	n.d.	n.d.	0.15	0.12	n.d.	n.d.
Cr ($mg \cdot l^{-1}$)	n.d.	n.d.	0.12	0.12	n.d.	n.d.
Cu ($mg \cdot l^{-1}$)	n.d.	n.d.	0.35	0.35	n.d.	n.d.
Fe ($mg \cdot l^{-1}$)	0.49	0.96	716	716	4.0	2.1
K ($mg \cdot l^{-1}$)	1.05	2.86	2.66	2.95	5.01	4.60
Mg ($mg \cdot l^{-1}$)	5.53	14.8	59.6	60.3	52.6	53.6
Mn ($mg \cdot l^{-1}$)	n.d.	n.d.	1.68	1.66	0.26	0.07
Na ($mg \cdot l^{-1}$)	5.43	8.00	7.39	4.54	50.7	54.3
Ni ($mg \cdot l^{-1}$)	n.d.	n.d.	0.70	0.67	n.d.	n.d.
Pb ($mg \cdot l^{-1}$)	n.d.	n.d.	0.034	0.033	n.d.	n.d.
Sr ($mg \cdot l^{-1}$)	0.08	0.28	0.27	0.28	0.58	0.62
Zn ($mg \cdot l^{-1}$)	n.d.	n.d.	6.22	6.21	n.d.	n.d.

Blanks were analysed together with the samples, and values remained always lower than detection limits for all the considered elements. For each analysed sample, the final concentration results from the average of three measurements.

Carbonates contents do not appear in the table because they were always below the detection limit.

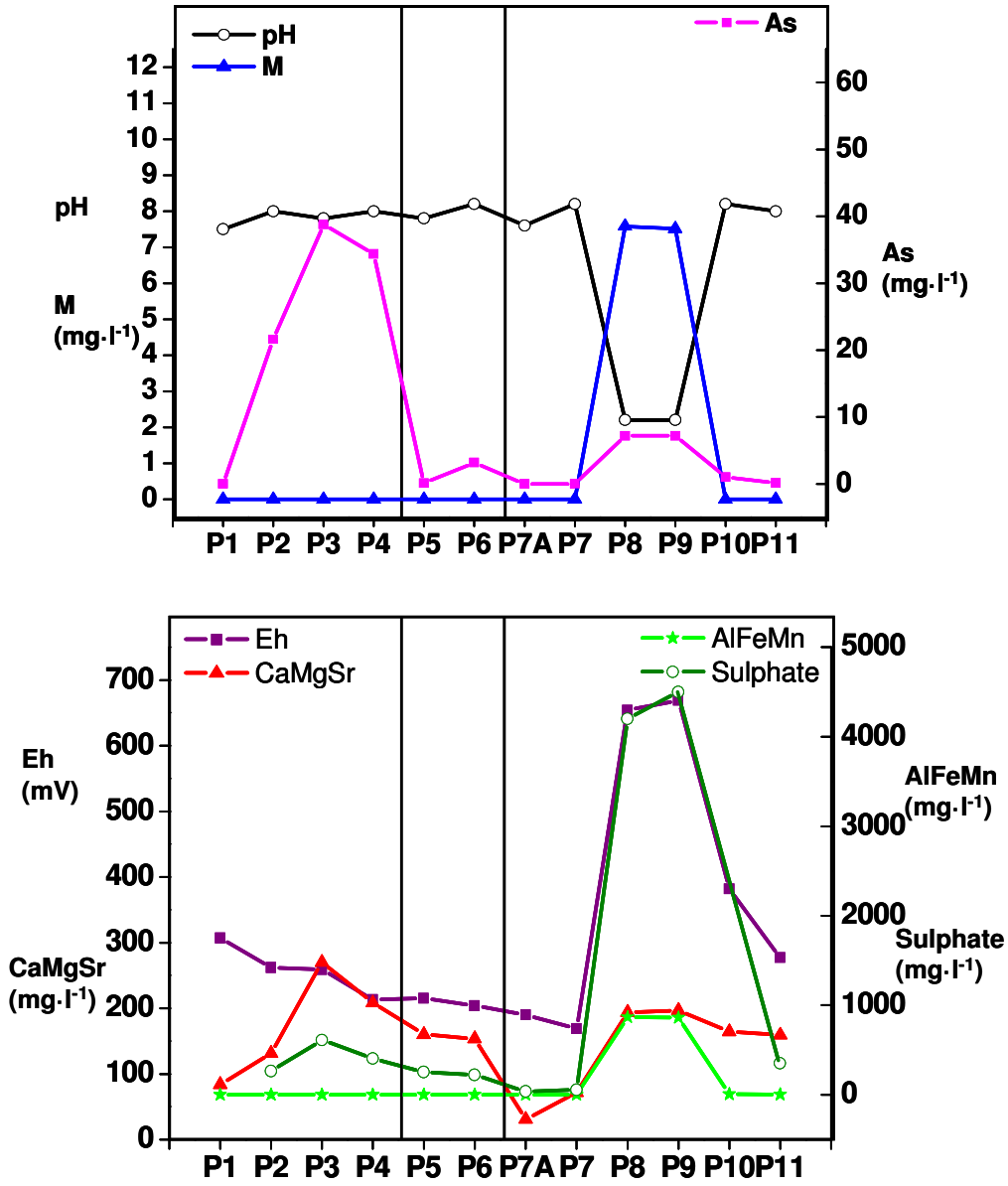

Fig. 5.1.3. Differences of the main parameters affecting arsenic release in the sampling points. "M" is the sum of Cd, Co, Cr, Cu, Ni, Pb and Zn concentrations (in mg·l^{-1}). "AlFeMn" is the sum of dissolved Al, Fe and Mn concentrations (in mg·l^{-1}). "CaMgSr" is the sum of Ca, Mg and Sr concentrations (in mg·l^{-1}).

Waters upstream and downstream of La Peña-El Terronal mine seem not to be so different, excepting for the higher arsenic concentration in P6. The point collected upstream the tailings (P5) presented the highest arsenic concentration among all points collected upstream in the studied mines (0.12 mg·l^{-1}). That means that in this site the presence of arsenic in surrounding waters may be in part due to the mineralogy of the area. In contrast, P6 was the sample presenting the lowest arsenic content of the points collected immediately after all mines (3.16 mg·l^{-1}). However, this concentration still remains being very high, which means that although the preventive measure carried out in this mine results in a marked decrease of arsenic release, it is not

enough. In addition, the water flow in this point (110 $l \cdot s^{-1}$ as mean value) is significantly higher than the rest of the points. In consequence, the input of arsenic to the surrounding waters is extremely high and very striking. No AMD occurred in this mine, as indicates pHs (7.80 – 8.2) and Eh values (204 – 215 mV). Hence, the solubilisation of heavy metals did not occur and conductivity values (886 – 1105 µS cm^{-1}) and salinity (0.42 – 0.54 ppt) remained not elevated.

In Los Rueldos mining site the influence of AMD is evident. In points collected upstream the spoil heap arsenic concentrations are non detectable, waters are weakly alkaline (pH= 7.9 – 8.6), moderately oxidant (Eh= 169 – 190 mV) and do not present detectable heavy metals concentrations in solution. However the situation in P8 and P9, just close to the spoil heap, changes drastically. The mineralogy of the area, with abundance of pyrites, together with a very strong oxidant redox potential (654 – 669 mV), cause the oxidation of sulphurs to sulphates (4200 – 4500 $mg \cdot l^{-1}$) resulting in an important decrease in pH (2.2). This lead to the solubilisation of heavy metals (the sum of the concentrations of Cd, Co, Cr, Cu, Ni, Pb, Zn ranges from non detectable upstream to 7.5 $mg \cdot l^{-1}$ in points downstream the spoil heap), as well as the arsenic contained in sulphur minerals, that reach values of 7.2 $mg \cdot l^{-1}$. The same occurs with Al, Mn and Fe, whose concentration as sum of the three elements increase from 0.49 and 1.14 $mg \cdot l^{-1}$ in P7 and P7A to 862 and 868 $mg \cdot l^{-1}$ in P8 and P9 respectively. Arsenic is usually adsorbed onto the surfaces of Al, Mn and Fe minerals [48, 49]. Therefore, the dissolution of these minerals provokes the release of arsenic to waters. The higher calcium and magnesium concentrations in waters that have passed through the tailings indicate that the solubilisation of calcium and magnesium arsenates is also responsible of the leaching of arsenic, although in less extent. Conductance (up to 5523 $\mu S \cdot cm^{-1}$) and salinity (up to 39.3 ppt) are the highest values for all the tested points, due to the great concentration of ions in solution.

For points P10 and P11, downstream the mine, it seems that the tested parameters tend to reach the values found upstream, indicating that the grade of pollution in waters decrease with the distance to the focus. However, arsenic concentrations is these two points (1.00 and 0.13 $mg \cdot l^{-1}$ respectively) still remain at elevated levels according to the Spanish law, that establish the limits in 500 $\mu g \cdot l^{-1}$ for mining and industrial effluents and 50 $\mu g \cdot l^{-1}$ for waters destined to production and consumption.

Fig. 5.1.3 highlights the differences in the main parameters affecting arsenic releasing in all sampling points. The similar behaviour of some variables can be

noticed. Eh increases from upstream (P7 and P7A) to points close to the spoil heap (P8 and P9) and then becomes lower again downstream (P10 and P11). The same tendency follow the amount of dissolved arsenic, metals concentration in solution (M, as the sum of Cd, Co, Cr, Cu, Ni, Pb, Zn concentrations), the sum of dissolved (Al+Fe+Mn), the sulphate concentration, and in less extent the amounts of (Ca+Mg+Sr). Just the opposite happens with pH, that decreases from upstream (P7 and P7A) to points close to the spoil heap (P8 and P9) and then becomes higher again (P10 and P11). This justifies that the release of arsenic into waters from Los Rueldos is the combination of the previously mentioned factors: the oxidation of sulphur minerals containing arsenic, the dissolution of Al, Mn and Fe minerals where arsenic was adsorbed to, and in less extent, the solubilisation of calcium and magnesium arsenates.

5.1.3.2 Statistical analysis

Due to the high number of parameters monitored in this study, it was difficult to establish relations between them. In this sense, statistical analyses together with chemometry are essential disciplines to tackle these studies [50, 51]. A statistical processing of the data was carried out by using SPSS software, version 11.5 and STATGRAPHICS Plus version 5.0 for Windows®. Supervised Pattern Recognition was applied to the physicochemical results at the three mine sites classified by their sampling points. Discriminant Analysis was performed with the 167 samples collected in the 12 sampling and Al, As, Ca, Fe, K, Mg, Na, Sr, Zn, electrical conductivity, Eh and pH results were considered as variables. This procedure generates a small number of functions of quantitative measurements which are linear combinations of the standardized pattern variables with weight coefficients. These functions are called “Canonical Discriminant Functions” and help to discriminate among groups of samples. The procedure assumes that the variables are drawn from population with multivariate normal distributions and that variables have similar variances. A graphical representation of the samples as a function of the two canonical discriminant functions is presented in Fig. 5.1.4. a, where F1 represents 88% of the variance and F2 represents 8.0%, both of them representing 96% of the total variance. These functions with P-values less than 0.05 are statistically significant at 95% confidence levels. The projection of the significant variables is represented in Fig. 5.1.4. b. Sampling point was used as grouping variable, where each category corresponds to the samples taken in the same point. Each sampling point is characterized by a centroid (marked as a +) which is the average for each category (unique value in the classification factor field). The graph shows that samples of a same

sampling point are closely grouped and there are not anomalous patterns (or outliers). Given the similar water composition found for some points, samples from points 2, 3 & 4 are grouped together, as well as 7 & 7A points, 8 & 9 and 10 & 11. Finally, samples were classified in six groups: those taken upstream and downstream of each mine site. In the figure, arrows represent the alteration of natural waters related to abandoned mine works and spoil heaps.

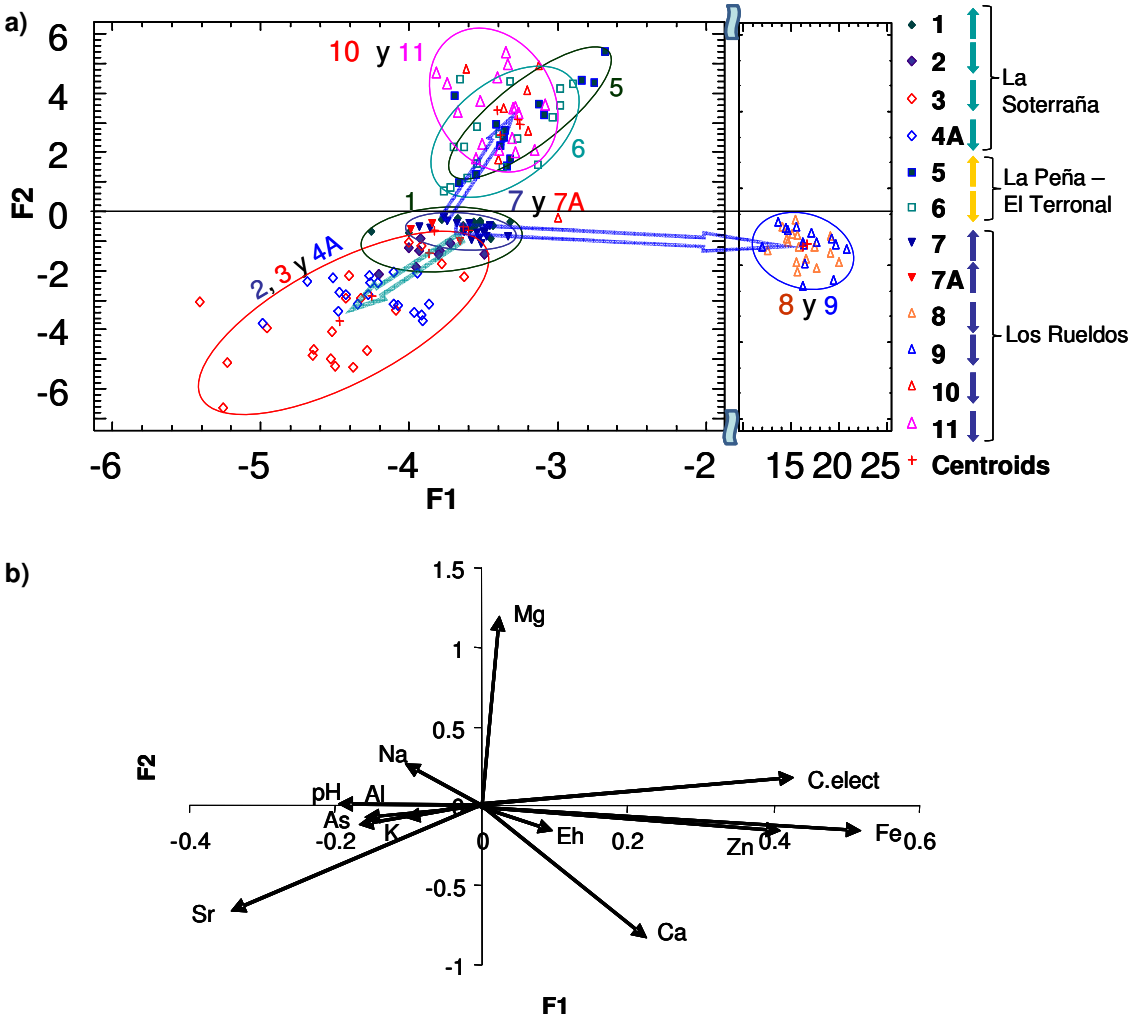

Fig. 5.1.4. a) Linear discriminant analysis of physicochemical data from La Soterraña, La Peña-El Terronal, and Los Rueldos mine sites. b) Representation of the contribution of the considered parameters to the functions F1 and F2.

This study reveals significant differences between samples taken upstream and downstream La Soterraña mine site, mainly as a consequence of its high enrichment in As, leached from the mineralized area and spoil heaps. Waters from La Soterraña have a noticeable different behaviour from the other mine sites. Ca, As, K, Na, Mg, Sr and pH values increased from sample point P1 to P2 and especially to P3 & P4 points, as a consequence of the presence of spoil heaps.

La Peña – El Terronal mine site does not cause a great influence due to the encapsulation of the area (both groups are located in the graph in the same zone with a large common area).

The influence of the Los Rueldos Mine was very strong in waters collected in 8 & 9 points, characterized by low pH, high concentrations of heavy metals and high electrical conductivity, but when these waters are incorporated to streams (10 & 11 points), this AMD typical behaviour changes quickly and it is neutralized. These statistical results corroborate the conclusions previously obtained.

Correlations among arsenic and other variables were established. The most remarkable correlations are summarized in Table 5.1.3.

Table 5.1.3. Pearson correlation coefficients among As and some selected variables (n=171).

Variables	P1	P2&P3&P4A	P5	P6	P7&P7A	P8&P9	P10&P11
Al	0.996[a]	-0.015	-0.239	-0.412	0.057	0.314	-0.152
Ca	-0.030	0.619[a]	0.099	0.774[a]	0.122	-0.375[b]	0.241
Fe	0.492	-0.082	-0.192	-0.372	0.059	0.621[a]	-0.107
Mg	-0.518	0.441[a]	0.165	0.677[a]	0.075	-0.335	0.246
Mn	0.985[a]	-0.094	-0.388	-0.349	-0.110	-0.173	-0.094
Sr	-0.275	0.425[a]	0.197	0.404	0.055	0.656[a]	-0.229
Bicarbonate		-0.207	0.320	0.845	-0.483	0.658	-0.214
Sulphate		0.643	0.279	0.937[b]	0.446	0.687	-0.424

[a] Statistically significant correlation at the 95% confidence level (two-tailed).
[b] Statistically significant correlation at the 99% confidence level (two-tailed).

The high correlation coefficient existing among As and Al concentrations in P1 (R = 0.996) seems to indicate that both elements are strongly associated in La Soterraña mine site. However, the no correlation of these elements in points downstream the mine points out that the leaching of arsenic does not occur due to the solubilisation of Al minerals where arsenic could be adsorbed to its surface. This does not happen either in La Peña mine. The correlation in the group of points P8 & P9 (R= 0.314) reveals that the presence of arsenic in these points could be in part due to the previously mentioned phenomenon. Certain similarity exists with Mn. Despite the high correlation between As and Mn contents in P1 (R= 0.985), indicating the association of these elements in La Soterraña, the inexistent correlation downstream the mine suggest that the solubilisation of arsenates bound to manganese oxyhydroxides is not very important, neither in La Peña and Los Rueldos mines. Ca, Mg and Sr showed noticeable positive correlations for points downstream La Soterraña mine and La Peña mine, meaning that the releasing of arsenic in these two mines is greatly because of the solubilisation of Ca, Mg and Sr arsenates. In

the case of Los Rueldos only As-Sr correlation was significant, indicating that releasing of arsenic in partially owed to the dissolution of Sr arsenates. An opposite behaviour occurs with iron. While the correlation among As and Fe concentrations is significant in points affected by AMD from Los Rueldos (R= 0.621), the rest of tested points showed no significant As-Fe correlation. Therefore, the releasing of the arsenates bound to the surfaces of iron oxihydroxides is only important in points just close to Los Rueldos spoil heap. Surprising is the significant correlation found between arsenic and sulphate concentrations downstream all mines, with r values of 0.643, 0.937 and 0.687 for La Soterraña, La Peña and (P8&P9) from Los Rueldos respectively. That means that oxidation of sulphur minerals containing arsenic occurs in all mines. Nevertheless, the extent of this phenomenon is much higher in Los Rueldos, because of the most favourable conditions in this mine as higher Eh and evidenced by the greater amounts of sulphate generated in P8 and P9 (Table 5.1.3). Another interesting correlation was found among arsenic and bicarbonate concentrations in P6 (R= 0.845) and P8&P9 (R= 658) suggesting that leaching of arsenic must occur somehow together with carbonates solubilisation in La Peña and Los Rueldos. Although natural carbonates usually do not contain significant amounts of arsenic it has been demonstrated that arsenic may be trapped also by natural calcite [52].

5.1.3.3 Arsenic speciation studies

5.1.3.3.1 Preservation of Arsenic Speciation

The preservation of arsenic species in water samples is an indispensable method to avoid their changes during storage. It has been established that arsenic preservation is dependent on the sample matrix [53]. The distribution of As(III) and As(V) species depends greatly on the abundance of redox-active solids, especially organic carbon, the activity of micro-organisms, and the extent of diffusion of O_2 from the atmosphere. Filtering the sample removes most of the colloidal material and micro-organisms that can affect the dissolved As(III/V) ratio [54, 55]. Since it has been demonstrated that similar effectiveness of filtration through 0.45 µm and 0.1 µm pore size is obtained to preserve As(III) [56], filtration by 0.45 µm is enough to keep arsenic speciation. On other hand, photochemical reduction of Fe(III) can provoke the oxidation of As(III) to As(V) [57] when a water sample containing Fe(III) at pH>2 is exposed to light. Hence, in order to prevent photochemical reactions that can affect the arsenic redox distribution [57, 58] samples should be stored in dark until analysis. Furthermore, acidification prevents oxidation and precipitation of Fe and Mn hydroxides that can coprecipitate or adsorb arsenic [59]. HCl acid has been proved to be the best inorganic

acid for this task [56], but also ethylenediamine tetraacetic acid (EDTA) has been reported as a good alternative to avoid the alteration of the original arsenic distribution, since this reagent chelates Fe and other metals and the formation of precipitates is inhibited [12, 60, 61]. Therefore, according to recommendations from literature, samples were filtered though 0.45 µm and HCl acid and EDTA were essayed as preservatives of samples as well as they were kept in dark until analysis.

The results of arsenic speciation carried out after the sample collection for the three different aliquots (without any additive, with HCl and with EDTA) show no significant differences between them. Results from the temporal study confirm that no alteration of the original arsenic distribution occurred up to nine months after the collection in any of the essayed media. These results are in agreement with those reported by some authors [62, 63], who found that HCl acidification and storage in the dark successfully preserved arsenic redox species, and also with the ability of preservation of EDTA [12].

In relation to preservation of total dissolved arsenic with time, reduction of the any species was not observed for any of the three aliquots along the considered time. This can be due to the fact that the main responsible in adsorbing arsenic and therefore lowering its concentration in solution are Mn and Fe, and their contents in the studied samples were very low, with the exception of P8 and P9 from Los Rueldos, whose contents rise up to more than 700 $mg \cdot l^{-1}$ for Fe and 1.68 $mg \cdot l^{-1}$ for Mn. However, neither a decrease in dissolved arsenic happened to these samples, even in the aliquot without preservation. The reason could be the nature itself of these samples, since their acid pH (mean value of 2.2) would avoid the precipitation of Mn or Fe compounds and the subsequent adsorption of arsenic into them.

5.1.3.3.2. Arsenic speciation

The results of arsenic speciation showed that only inorganic species were detected (Table 5.1.4). The absence of organic arsenic species reflects the origin of contamination by weathering of mine wastes containing arsenic minerals. Moreover, speciation results indicate that arsenic occurs exclusively as As(V) in most samples, which is in agreement with the theoretical Eh-pH diagram of species (Fig. 5.1.5).

Table 5.1.4. Arsenic species, pH and Eh upstream and downstream the three mine sites.

Sampling point		As(V) ($\mu g \cdot l^{-1}$)	As(III) ($\mu g \cdot l^{-1}$)	pH	Eh (mV)
La Soterraña	P1	56		7.5	307
	P2	24000		8.0	262
	P3	37250		7.8	259
	P4A	37500		8.0	213
La Peña	P5	182	5	7.8	215
	P6	2475	77	8.2	204
Los Rueldos	P7A	13		7.6	190
	P7	9.8		8.2	169
	P8	7125		2.2	654
	P9	7350		2.2	669
	P10	176		8.2	382
	P11	65		8.0	277

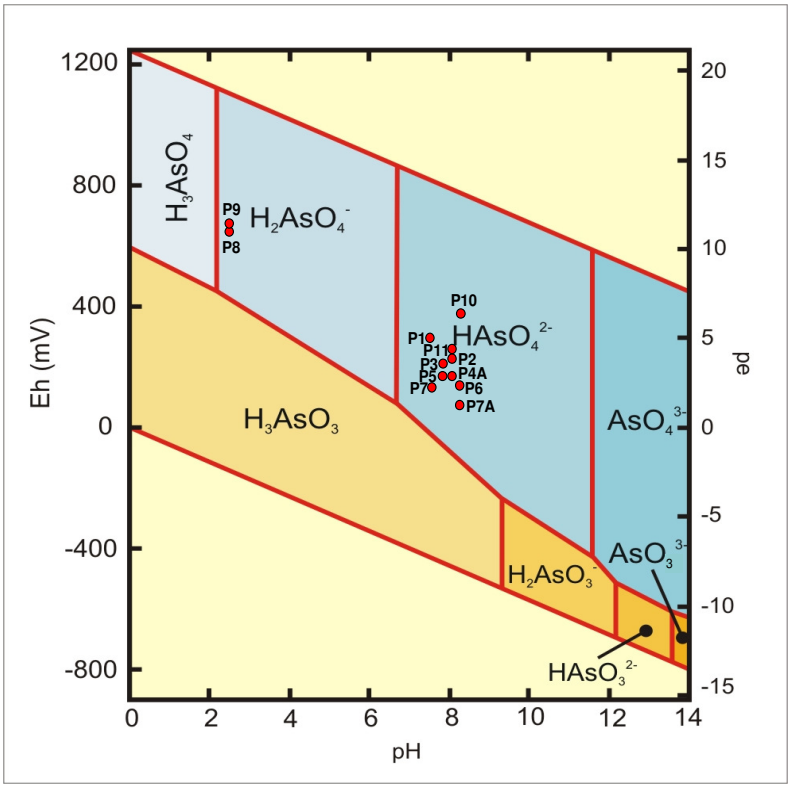

Fig. 5.1.5. pH-Eh diagram for arsenic speciation with the location of studied points.

It has been described that the presence of high As(V) concentrations can be explained by the promotion of desorption of arsenates bound to iron oxyhydroxides at neutral-alkaline conditions [64] as well as dissolution of thermodynamically unstable Fe and Ca-arsenates minerals [65, 66]. Taken into account the low Fe concentrations present in La Soterraña samples and its relatively high Ca and Mg concentrations, it seems that dissolution of Ca and Mg arsenates must be the most important process controlling As(V) releasing into waters in this area. In the case of samples P8 and P9 from Los Rueldos, affected by AMD, the high Fe concentrations suggest that

dissolution of adsorbed arsenates onto iron oxyhydroxides is the predominant process, together with dissolution of Ca and Mg arsenates in a less extent. In contrast to samples from La Soterraña and Los Rueldos, low concentrations of As(III) were detected in samples from La Peña, with proportions of As(III) around 3%. On the contrary to samples from La Soterraña and most from Los Rueldos, samples P5 and P6 showed high dissolved organic contents (20.3 and 17.9 $mg \cdot l^{-1}$ respectively). The potential of dissolved organic matter (DOM) to chemically reduce arsenates to arsenite has been studied by other authors [67]. The redox properties of natural DOM are not accurately known but the experiences with model compounds suggest that DOM can cover a wide spectrum of redox potentials. In addition, thermodynamic calculations suggest that reduction of arsenate with DOM is an energetically favoured process [67]. The rate of arsenite appearance was comparable to that obtained by Bauer and Blodau (2006) [68] in aqueous phase of soil samples.

5.1.3.4 Seasonal variations of total arsenic and some associated parameters

A seasonal variation study was carried out in selected samples collected every three months for a complete year in each mine site. Although it would be desirable to have sampled waters before and after the spoil heap in each mine, it was not possible in the case of La Soterraña, as no water flow was found in P1 during the whole studied year. Relationship between arsenic concentration in the studied waters and period of the year was intended to be studied. The other studied parameters were pH and redox potential, which are the main variables influencing arsenic mobility and speciation. These variations are presented in Fig. 5.1.6 a, b and c, whereas water flow values are summarized in Table 5.1.5. Hence, only the downstream points (P3 and P4A) were selected for this study. From La Peña-El Terronal, both upstream and downstream points are shown (P5 and P6). In Los Rueldos, sample P7 was chosen for representing the area upstream the spoil heap and P11 as a point downstream the mine. Moreover, P9 was included in this investigation due to the special characteristics of water influenced by AMD. In this point water is stagnant.

Table 5.1.5. Water flow in the studied points every sampling campaign.

Period	Water flow ($l \cdot s^{-1}$)					
	P3	**P4A**	**P5**	**P6**	**P7**	**P11**
Dec´09	0.010	0.300	87.2	99.8	0.010	0.005
Mar´10	0.025	0.041	155.3	162.9	0.008	0.001
Jun´10	0.040	0.052	121.9	130.4	0.005	0.001
Sep´10	0.020	0.025	84.6	95.4	0.015	0.000

Note: Water course is often dried in P1 and it is stagnant in P9.

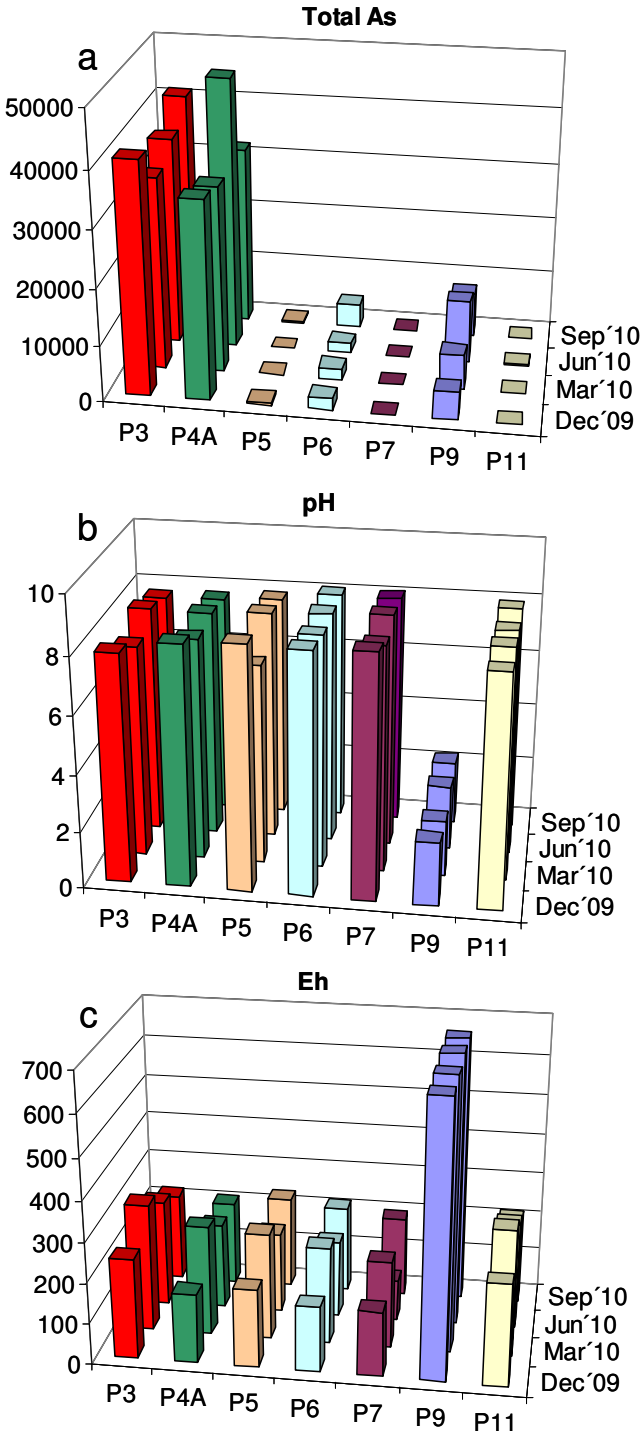

Fig. 5.1.6. Seasonal variations in a) arsenic contents ($mg \cdot l^{-1}$), b) pH and c) Eh (mV) upstream and downstream each mine site.

For La Peña and Los Rueldos mining areas, the highest values of arsenic concentration coincided with the lowest values of water flow in the sampling points (Fig. 5.1.6 a). It is noticeable that point P6, downstream La Peña, showed an average arsenic concentration of 2.6 $mg \cdot l^{-1}$ with an average water flow of 122 $l \cdot s^{-1}$. This means an average input of total arsenic of 0.3 $g \cdot s^{-1}$ to San Tirso River, occurring the most important contribution during lately summer (0.4 $g \cdot s^{-1}$). Among studied areas, points

from La Peña presented the highest water flow values since they are collected from a river close to the spoil heap. This arsenic input to waters is relatively high compared with other studies developed in other mining sites. Hewlett et al. (2005) [69] reported arsenic inputs below 2 $mg \cdot s^{-1}$ in river waters from adjacent coal and gold mines. However, these arsenic inputs are lower than those reported by Masson et al. (2007) [70] who found arsenic discharges above 3 $g \cdot s^{-1}$ into the Gironde estuary receiving water from historic mining areas in France. De Gieter et al. (2005) [71] reported arsenic discharges of 0.2-1.1 $g \cdot s^{-1}$ in the Scheldt estuary from drains of both industrial and domestic wastes.

pH values remained approximately similar for all tested sampling points during the observation period (Fig. 5.1.6 b). For most points the lowest values corresponded to samples collected during March. The decrease of alkalinity can be attributed to the dilution effect due to the rainfall between December and March. On the contrary, effect of rainfall resulted no significant for point P9. This is consistent with results obtained by other authors for natural waters affected by AMD [26].

Related to Eh (Fig. 5.1.6 c), the highest values were found in samples collected during early spring (March) for all mine sites. This behaviour is expected since the increasing of water flow occurs together with an increasing of dissolved oxygen.

Nevertheless, no remarkable seasonal variations could be established for any of the studied variables in the three mines, contrary to what occur with other rivers [26].

5.1.4 CONCLUSIONS

Results from this study confirmed the high degree of As pollution in waters from the studied mining areas. It is evident that mine tailings act as a continuous source of arsenic mobilization into water. However, the environmental impact of mining is different for the three studied mine sites, and in accordance with the mineralogy, the geological and the physicochemical characteristics of each area. The highest total arsenic concentrations were found in waters from La Soterraña mining area (up to 38.8 $mg \cdot l^{-1}$). It may be explained by the massive solubilisation of calcium and magnesium arsenates, and in less extent by the solubilisation of strontium arsenates and the oxidation of sulphur minerals containing arsenic.

In La Peña-El Terronal, where the encapsulation of the main spoil heap was carried out, a lower impact in waters is expected. However, the high arsenic

concentrations found downstream reveal that the preventive measure is not enough to avoid arsenic releasing onto waters. In fact, the high water flow in this point implies the greatest input of arsenic to surroundings, an average of 0.3 $g \cdot s^{-1}$ of arsenic reach San Tirso River, which means that the arsenic spread in this mine site is the most important among the studied mines. arsenic leaching in this mine is due to the solubilisation of calcium, magnesium and strontium arsenates, as well as because of the oxidation of sulphur minerals containing arsenic and somehow it occurs together with carbonates solubilisation.

Waters close to Los Rueldos spoil heaps are strongly affected by AMD. This entails an important solubilisation of arsenic and heavy metals. In this case the release of arsenic may be attributed to different sources: the oxidation of sulphur minerals containing arsenic, the dissolution of Al, Mn and Fe minerals where arsenic was adsorbed to, and in less extent, the solubilisation of calcium, magnesium and strontium arsenates. Despite the high concentrations of arsenic and heavy metals, quality of waters notably improves with distance to the focus. Anyway, arsenic concentrations exceed Spanish limits of 500 $\mu g \cdot l^{-1}$ of arsenic for mining and industrial effluents in most of tested points downstream the mines.

The presence of arsenic in the studied waters only occurs below the form of inorganic species, and almost exclusively as As(V), with the exception of a low amount of As(III) found in waters from La Peña-El Terronal.

Regarding the preservation of samples, no alteration of arsenic speciation was found up to nine months after collection for samples stored without any preservative, or for those preserved with HCl or EDTA acids.

In contrast to other rivers, no important seasonal variations in the main physico-chemical parameters controlling arsenic concentrations and speciation were observed in the three studied mining areas, which means that releasing arsenic remains approximately constant along the year and the dependency of rainfall is not very remarkable.

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6. ARSENIC IN PLANTS FROM MINING ENVIRONMENTS

This chapter completes the study of the impact of arsenic pollution in mining environments with the evaluation of the arsenic contamination suffered by plants growing in those areas.

The extent of arsenic contamination in the tissues of certain plant species from two mine sites will be determined. Likewise, the dependency of the degree of arsenic accumulation by plants on the plant species in question will be evaluated, as well as the possible dependency on the level of contamination of their corresponding soils. This involves the assessment of the bioavailable arsenic fraction in soils for plants uptake and the study of arsenic transference from soils to plants. In this sense, it will be take advantage of the usefulness of the proposed sequential extraction protocol since the two most labile fractions of that protocol could be considered suitable for an estimation of the soil arsenic fraction potentially available to plants.

Similarly to the other considered matrices, the study of the forms of occurrence of arsenic will be also studied in plant tissues with the aim of evaluating the impact of arsenic contamination in plants in terms of toxicity.

The extraction of arsenic species from plant tissues without altering the original speciation and avoiding interconversion or losses during the extraction process is essential. An appropriate methodology for the extraction of the most important arsenic species in terrestrial plant materials (As(III), As(V), MMA, DMA) will be developed and optimized. It must be convenient for highly polluted samples, as those studied in the current research. In this case, the speciation analysis of plant samples will be performed by means of the hyphenated techniques HPLC-ICP-MS.

6.1 A methodological approach to evaluate arsenic speciation and bioaccumulation in different plant species from two highly polluted mining areas

ABSTRACT

Arsenic accumulation and speciation in different plant species growing in two polluted sites were examined, in addition to arsenic transfer from soils to plants. The studied areas were two abandoned mercury mines in Northern Spain, La Soterraña and Los Rueldos. Plants accumulated extremely high amounts of arsenic in their tissues. Accumulation rates depend on both the grade of pollution of the site and on the plant species itself. Total arsenic concentrations varied depending on the part of the plant, with roots accumulating the most arsenic in all the studied plants (up to 1400 $mg \cdot kg^{-1}$). A suitable extraction method for arsenic speciation in the studied samples was developed and optimized using 0.3 M orthophosphoric acid as the extracting agent in a graphite block digestion system at 90 ºC for 1 h. Extraction efficiencies were above 80% and speciation was not altered, since recoveries of the spiked arsenic species (As(III), As(V), DMA (dimethylarsonic acid) and MMA (monomethylarsonic acid)) were between 94 and 102%. Speciation studies were performed on the parts of each plant species by means of HPLC-ICP-MS with the results indicating no significant differences in the speciation between parts of the plants. As(V) was always the predominant species, although a non-negligible amount of As(III) was found for all samples. Little amounts of MMA and DMA occurred in certain samples. Arsenic uptake by plants depends on the plant available arsenic fraction in soils, here considered as the sum of water-soluble and phosphate-extractable fractions. Although soils from La Soterraña were less polluted, arsenic bioavailability was much higher than in Los Rueldos. Soil-to-plant transfer factors, based on total and available arsenic in soils, were evaluated for the studied plants. Dryopteris filix-mas and Calluna vulgaris (L.) *Hull*, from La Soterraña and Los Rueldos respectively, were the plants with the highest abilities to extract arsenic from their corresponding soils.

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6.1.1 INTRODUCTION

Arsenic (As) is a metalloid commonly found in nature, being the twentieth most abundant element in the earth's crust [1, 2]. Its presence can have a natural origin, like the mineralogy of the area, or can be due to anthropogenic sources, such as mining activities [3]. Ore processing generates a considerable amount of gases, aerosols and particulate matter containing metals that can been fixed by sediments, soils or biota [4]. Furthermore, the waste materials are located along the mining districts and natural weathering of these tailings can mobilize pollutants to the surroundings. Hence, although normal arsenic levels in soils range from 1 to 40 $mg \cdot kg^{-1}$, these levels are significantly higher in mining soils [2, 5]. Likewise, whereas arsenic concentrations in plants growing on uncontaminated soils vary from 0.009 to 1.5 $mg \cdot kg^{-1}$, being leafy vegetables in the upper range and fruits in the lower range, plants growing in polluted areas can accumulate extremely high amounts of arsenic, depending on the location and pollution source [6]. In fact, values up to 6000 $mg \cdot kg^{-1}$ have been reported for arsenic in plants grown in mining sites [7].

Toxicity, biological availability and transport mechanisms of arsenic highly depend on its chemical speciation [8]. It is well known that inorganic forms are more toxic and mobile than organoarsenic species and among them arsenite (As(III)) is considered to be more toxic and mobile than arsenate (As(V)) [9, 10]. It has been reported that As(III) was 10 times more toxic than As(V) and 70 times more toxic than monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) [11, 12].

In terrestrial plants arsenic is predominantly present in the form of inorganic species, although organic species have been identified as well [13]. The main problem in the study of arsenic speciation in plants still lies in the establishment of a convenient extraction technique avoiding contamination, losses and transformation of species [14]. The diversity in the extraction methods indicates that there is no agreement between authors on the choice of the best extraction conditions [15]. A desirable extraction method should quantitatively extract all the arsenic species without altering their original speciation and therefore, mild extraction conditions are required. For this purpose, different solutions have been used. Among them, mixtures methanol-water (at different percentages of each one) [16, 17] and orthophosphoric acid (at different concentrations) [18] have been the most popular.

The determination of available arsenic species for plants grown in polluted soils is important for understanding the uptake, transfer and accumulation processes of

arsenic in plants [19]. Total arsenic concentration in soils is not a good indicator of mobility, availability and the associated environmental risk [20-22]. The main difficulty on the study of arsenic availability for plants relies on the evaluation of an appropriate extraction method for soils, since it is desirable that the applied method simulates the real uptake by plants. Extraction in one step with certain saline solutions or suitable sequential extractions have been proposed as good alternatives to total concentration measurements [23-26]. Phosphate has been proven as a good predictor of arsenic bioavailability for plants [27]. Due to its chemical similarity to arsenate, phosphate competes with it for soil adsorption sites [28-30]. Hence, the use of phosphate as an extractant agent reflects better the arsenic bioavailable portion. In fact, good correlations have been reported between arsenic contents in some vegetables and their corresponding phosphate-extractable fraction in soils, rather than with total soil arsenic [27].

The transfer of arsenic from soils to plants has been evaluated by means of soil-to-plant transfer factors. The transfer factors depend not only on the plant species, but also on the arsenic concentration and its availability in the soil. Kloke et al. (1984) [31] reported that the typical soil-to-plant transfer factors for arsenic ranged from 0.01 to 0.1 for normal plants. However, this value should be greater than 1 for hyperaccumulator plants [32-34].

Little is known about the mechanisms of uptake and distribution of arsenic along the parts of the plants. It is generally assumed that roots are responsible for accumulating the most arsenic [18, 35], although hyperaccumulator plants are able to translocate the greater part of arsenic to the aerial part [36]. The largest proportion of arsenic taken up by plants is in the form of inorganic As(V), since in aerobic soils arsenate is the most stable and dominant species. Plants are able to reduce arsenate to arsenite. The mechanism of this reduction has been studied in hyperaccumulator plants and it has been proposed that when the plant is exposed to arsenic, under the reducing conditions of plant cells, As(V) is readily reduced to As(III) and complexed by organic ligands such as thiols, to avoid damage to plant cells [37, 38]. However, it is not well known whether the organic arsenic compounds found in plant tissues are simply taken up as such from soil or whether they were converted from inorganic species in soils to methylated forms in the plants [39]. Transformation and translocation of arsenic compounds among parts of the plant highly depends on plant species and soil characteristics [20]. It has also been determined that plants take up the arsenic from the soil through the phosphate transport systems [40-42] and that the reduced

arsenic translocation and accumulation in non As-hyperaccumulator plants may be attributed to suppression of the high-affinity phosphate uptake system [43].

The main objectives of this work were i) the establishment of an appropriate extraction method for arsenic in plants with very high arsenic contents; ii) the study of arsenic accumulation and speciation in differentiated parts of several plant species grown in two mining sites; iii) the assessment of the arsenic transfer from soils to plants by means of transfer factors.

6.1.2 MATERIALS AND METHODS

6.1.2.1 Study site

Plants from two mercury mining districts, La Soterraña and Los Rueldos, in Asturias, Northern Spain, were collected and studied.

La Soterraña (Fig. 6.1.1 a) is located close to the village of Muñón Cimero (Pola de Lena district), within the scenic valley of a tributary of the Caudal River, which is one of the most important rivers in the region [4]. The mine exploited an epigenetic-type ore deposit mainly constituted by cinnabar, orpiment, realgar, pyrite (usually with high arsenic concentrations), arsenopyrite, marcasite, and pararealgar, in a gangue of quartz and calcite [44]. From an environmental point of view, the presence of arsenic minerals in the ore deposit paragenesis is especially significant, as high arsenic concentrations have been found in pyrites and iron oxides. Currently, the legacy of the historical mine works at La Soterraña remains in the form of old industrial facilities and a big spoil heap of about 17,000 m^2 with significant quantities of wastes from mine and metallurgical operations. The wastes were placed in the neighborhoods of the mine, in an area where the abrupt topography and drainage are important factors favoring the ore weathering and consequent mobilization of metals and metalloids [4].

Los Rueldos mining works and the associated spoil heap are located along the northwestern slope of the valley of Morgao stream, about 2 km northeast of the town of Mieres and 20 km southeast of Oviedo, the capital city of Asturias (Fig. 6.1.1 b). The epigenetic deposit contains Hg mainly as cinnabar. Other primary metallic minerals which are present in the paragenesis of the ore are pyrite, occasionally As-rich pyrite, melnikovite, sphalerite, marcasite, chalcopyrite, arsenopyrite, galena, stibnite and realgar. Smithsonite, hemimorphite, cerusite, goethite, malachite, jarosite, melanterite, scorodite and gypsum are present as secondary minerals. The more conspicuous

environmental legacies of the mining activities at the site of Los Rueldos remains in the form of mine wastes, totalizing around 3000 m^3, lying on the mountain side downhill from the mine entrances. The spoil heap has an irregular, long shape and it extends in an area of 1340 m^2. The steep slope of the spoil heap (38% average) and the humid climatic conditions favor the weathering of sulfide-rich rocks promoting vigorous mechanical and chemical dispersions, and all contribute to the spread of the ecotoxic elements to the environment [45].

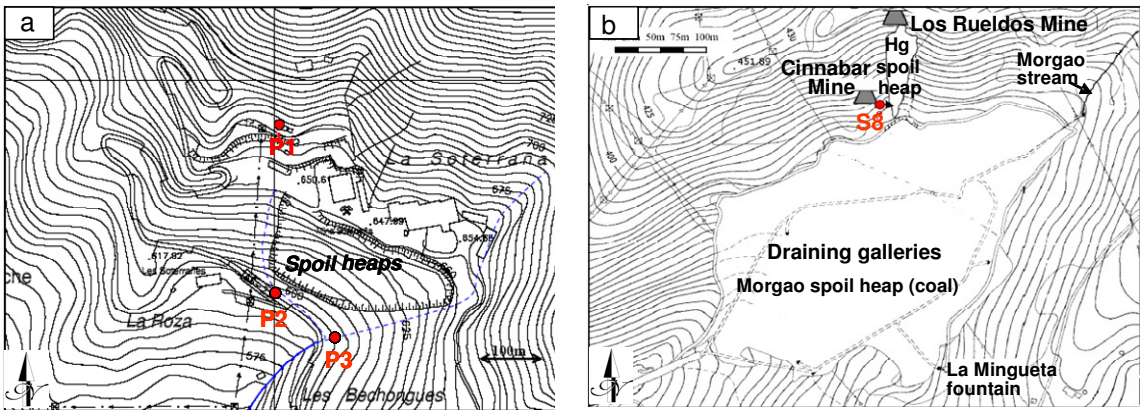

Fig. 6.1.1. Map of the abandoned Hg mine sites and sampling points: a - La Soterraña mine and b - Los Rueldos mine.

6.1.2.2 Plants and soils sampling and preparation

The most abundant plant species were collected from the studied mining sites, as well as their corresponding soils. In La Soterraña two points were sampled (Fig. 6.1.1 a). P1 was located upstream of the waste piles. *Crupina vulgaris Cass.* was the plant species collected here. P3 was close to a stream at the bottom of the spoil heap and receives its leachates. Three plant species were sampled there: *Equisetum telmateia Ehrh.*, *Typha latifolia* and *Dryopteris filix-mas.* In Los Rueldos three different plant species were collected from the same point, P8, situated next to a small pool, where acid mine drainage flowing from an abandoned mine gallery is stored, after the Hg spoil heap (Fig. 6.1.1 b) . These were *Quercus robur L.*, *Calluna vulgaris (L.) Hull*, and *Dryopteris affinis*. After collection, each plant sample was vigorously washed with tap water and rinsed with deionized water. They were divided in aboveground mass and roots; leaves and stalks were separated when it was possible (as was the case for *Calluna vulgaris* and *Dryopteris affinis*. In *Calluna vulgaris* woody and radicle roots were also sorted. Then, they were air-dried for 7 days to ensure constant weight and afterward ground using the stainless steel mill “IKA A 11 Basic” to pass a 0.5 mm sieve.

Soils corresponding to each plant were collected at 25 cm deep when possible, and stored in polyethylene bags. Samples were disaggregated in the laboratory and the non-mineral material and rock fragments higher than 2 mm were discarded. Then, they were oven dried at a temperature below 40 ºC to minimize loss of volatile elements (7-15 days), sieved and ground in an agate mortar to a size finer than 63 µm.

6.1.2.3 Analytical methods

6.1.2.3.1 Reagents and standards

All standards and reagents were of analytical grade except for the orthophosphoric acid, nitric acid and hydrogen peroxide which were Merck Suprapur reagents. Stock solutions (As= 1000 $mg \cdot l^{-1}$) of arsenic species were prepared by dissolution of $NaAsO_2$ (Merck), $Na_2HAsO_4 \cdot 7H_2O$ (Prolabo), $CH_3AsO(ONa)_2 \cdot 6H_2O$ (Carlo Erba), $(CH_3)_2\ AsO_2Na \cdot 3H_2O$ (Stream Chemicals) in 18 MΩ·cm ultrapure water from a Milli-Q system (Millipore). These solutions were stored in the dark at 4 ºC and their stability frequently checked. As(III) standard solution was kept 1 month to prevent eventual transformation into As(V) species. The others solutions can be considered stable for several months. Intermediate standard solutions (As= 10 $mg \cdot l^{-1}$) were prepared daily and used directly for the preparation of appropriate working standard solutions which were tested every day in the quality control of the analytical procedure. Mobile phase was prepared by dissolving TRIS (hydroxymethyl aminomethane) (Aldrich) with ultrapure water and adjusting pH to 7 with glacial acetic acid (99.99 %, Aldrich). It was continuously filtered through a 0.45 µm membrane. Samples were filtered through a 0.2 µm nylon filter just before injection into the HPLC column. Selenium was used as internal standard. Solutions of Se(IV) and Se(VI) were prepared from ACS grade sodium selenite and sodium selenate from Sigma.

All containers were cleaned by soaking several days in a 10% HNO_3 solution and rinsed with ultrapure water before use.

6.1.2.3.2 Instrumentation

Total arsenic contents in plants were determined by Agilent 7500ce ICPMS instrument equipped with an octopole collision/reaction cell. Sample introduction system was constituted of a "MicroMist Nebulizer" and a Scott double pass spray chamber cooled to 2 ºC. According to Darrouzès et al. (2008) [46] parameter settings were performed as follows: Ar plasma gas flow, 15 $l \cdot min^{-1}$; Ar auxiliary gas flow, 0.86

$l \cdot min^{-1}$; Ar nebulizer gas flow, 1-1.1 $l \cdot min^{-1}$; H_2 gas flow, 4 $l \cdot min^{-1}$; radio frequency forward power, 1500W; nickel sampler and skimmer cones. These parameters were optimized daily using a solution of 1 $g \cdot l^{-1}$ of lithium, yttrium and thallium (1 $ng \cdot ml^{-1}$ in HNO_3 2%). Acquisition parameters were: integration time, 100 ms per isotope; 10 replicates; monitored m/z 75, 77, 78.

Total arsenic contents in soils, as well as arsenic in available fractions, were analyzed by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES), using a VARIAN 735-ES instrument with radial configuration.

Chromatographic separation of arsenic species in plant material was carried out using an Agilent 1100 series HPLC pump, equipped with an autosampler and a variable volume sample loop.

Acid digestions in soils and arsenic extractions in plants were performed by an Ethos One microwave system (Milestone, USA). Acid digestion and arsenic extractions in plants were carried out with a Digiprep Jr block digestor (SPS Science) equipped with a temperature-time programmable controller and polypropylene digestion tubes.

6.1.2.3.3 Determination of total arsenic in plants

Digestion of the parts of the different plant species was developed in a graphite heating block at high temperature (DP). 0.2 g of dry sample was weighed in a polypropylene tube and 5 ml of HNO_3 and 2 ml of H_2O_2 were added. The mixture was subjected to a temperature-time program using a temperature ramp to 90 ºC which it maintained for 4 h. After cooling, digested samples were made up with ultrapure water to 25 ml and filtered through 0.45 µm filter before analysis. Appropriate dilutions were made for the analysis. To ensure the quality of the results, the same procedure was applied to certified reference materials (CRM) of plants: (i) Virginia Tobacco Leaves (CRM-CTA-VTL-2), whose total arsenic content is 949 ± 72 $\mu g \cdot kg^{-1}$; (ii) Peach Leaves (CRM-GBW-08501), with a total arsenic concentration of 340 ± 60 $\mu g \cdot kg^{-1}$. The obtained results were 951 ± 10 and 344 ± 21 $\mu g \cdot kg^{-1}$ respectively, in good agreement with the certified values. Analysis were carried out by ICP-MS. Regarding calibration, the slope obtained for normal calibration was compared with those of standard addition method. Results indicated no matrix effects for all samples, so external calibration was used throughout this study.

6.1.2.3.4 Arsenic extraction in plants

According to Bohari et al. (2002) [18] a 0.3M orthophosphoric acid solution was selected to extract arsenic species from plants without altering its speciation. Two extraction procedures were compared: microwave assisted extraction (MW) and extraction in a graphite digestor block at high temperature (DP). Extractions were conducted with the plant species Calluna Vulgaris leaves material with an arsenic concentration of 359 ± 2 $mg \cdot kg^{-1}$. A mass of 0.2 g of dry sample was mixed with 25 ml of extracting agent in a polypropylene tube. After the extraction program, samples were allowed to cool to room temperature and centrifuged. After 20 min at 4000 rpm all the supernatants were diluted up to 50 ml. Following the recommendations given by Vergara-Gallardo et al. (2001) [47] and Bohari et al. (2001) [48] a tenfold secondary dilution was carried out to preserve the speciation. The samples were filtered through a 0.2 µm filter before chromatographic analysis. Digestion systems were programmed to heat the sample at a specific temperature and maintaining it for a specified time. Tested conditions for each procedure are included in Fig. 6.1.2.

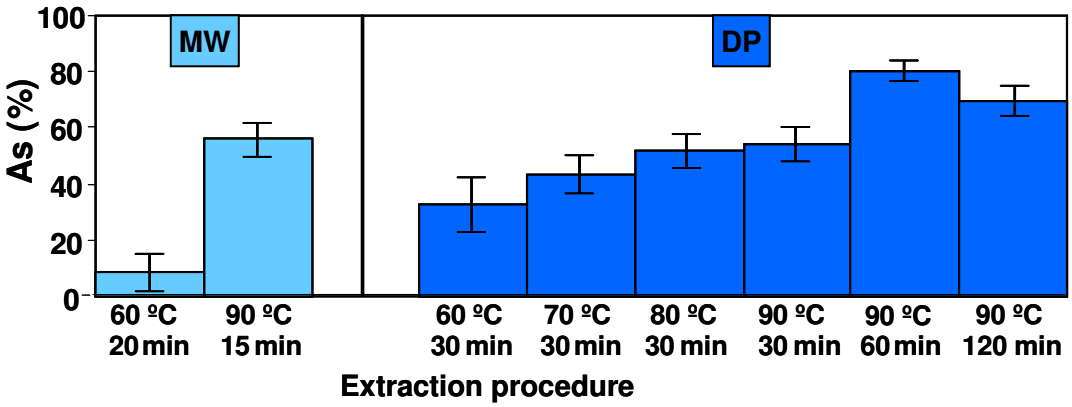

Fig. 6.1.2. Conditions and efficiencies of the different extraction procedures as % of arsenic extracted regarding total arsenic concentration. Error bars represent standard deviation for three replicates.

Although there is no reference material certified for arsenic speciation, the finally selected extraction method was also carried out with the CRM Virginia Tobacco Leaves CTA-VTL-2 to evaluate the analytical methodology. Total contents of the extracts were analyzed by means of ICP-MS.

6.1.2.3.5 Arsenic speciation in plants

The separation and determination of arsenic species in the orthophosphoric extracts was performed by HPLC-ICP-MS. The four more common arsenic species in plants (As(III), As(V), DMA and MMA) and the internal standard were completely

separated in a Hamilton PRP-X100 anion-exchange column (250x4.1 mm id 10 µm). The mobile phase and the elution program were selected and performed according to Milstein et al. (2002) [49]. Two TRIS acetate solutions, 30 and 100 mM, buffered at pH= 7, were used as eluent and the gradient elution program: from 0 to 7 min, ramp from 30 mM to 100 mM; from 7 to 12 min, hold at 100 mM; at 12.5 min, step to 30 mM. Flow rate: 1.5 ml·min^{-1}. Injected volume: 200 µl. All species were eluted in less than 13 min. Since the results achieved on speciation by external calibration agree with those obtained by standard additions method, it was enough to use external calibration.

6.1.2.3.6 Determination of total arsenic in soils

Total arsenic in soil samples was determined after microwave assisted acid digestion. 0.25 g of sample was weighed in a Teflon vessel and a mixture of 7.5 ml of HNO_3, 2.5 ml of HCl and 6 ml of HF was added. Resulting digests were transferred to Teflon capsules and 2 ml of $HClO_4$ were added and heated in a hot plate until evaporation of hydrofluoric acid, transferred into 25-ml volumetric flask, diluted with water to the mark, filtering when necessary and analyzed ICP-AES.

Assurance of the procedure was made with CRM NIST 2710 (Montana soil).Estimated errors were lower than 5%.

6.1.2.3.7 Determination of available arsenic in soils

Available arsenic was considered as the sum of water-soluble and phosphate-extractable fractions [50].

Water-soluble extraction: 0.5 g of soil sample was weighed in a polypropylene tube, 30 ml of ultrapure water were added and vortexed. After shaking 24 h at 35 rpm in an end-over-end shaker samples were centrifuged at 5000 rpm for 15 min. Supernatants were taken with Pasteur pipette, filtered through 0.45 µm pore paper filters and made up to 50 ml.

Phosphate-soluble extraction: 40 ml of 0.5M sodium phosphate at pH 8 were added to the solid residue obtained from the water-soluble extraction and shaking for 8 h. Again supernatants were taken with Pasteur pipette, filtered through 0.45 µm pore paper filters and made up to 50 ml.

The extracts were stored at 4 ºC until analysis by ICP-AES.

6.1.3. RESULTS AND DISCUSSION

6.1.3.1 Total contents and arsenic distribution in plants

The studied plants contained extremely high arsenic contents in their tissues, as it is shown in Table 6.1.1. The plant species with the highest amount of total arsenic was *Calluna vulgaris*, from Los Rueldos, with 359 $mg \cdot kg^{-1}$ of arsenic in its leaves, 150 $mg \cdot kg^{-1}$ in stalks and 280 and 1217 $mg \cdot kg^{-1}$ in woody and radicle roots respectively. It was followed by *Dryopteris affinis,* also from Los Rueldos, accumulating 77 $mg \cdot kg^{-1}$ of arsenic in its leaves, 46 $mg \cdot kg^{-1}$ in its stalk and 1371 $mg \cdot kg^{-1}$ in roots. From La Soterraña, the plant species having the greatest amount of arsenic were *Crupina vulgaris*, with 167 and 791 $mg \cdot kg^{-1}$ of arsenic in aerial part and roots respectively; and *Dryopteris filix-mas,* whose arsenic concentrations were 177 and 377 $mg \cdot kg^{-1}$ in aerial part and roots respectively. Total arsenic contents of the soils where plant species were collected are summarized in Table 6.1.2. It can be observed that the most polluted soils corresponded to *Calluna vulgaris*, with 25901 $mg \cdot kg^{-1}$ of arsenic, and to *Dryopteris affinis*, with 15423 $mg \cdot kg^{-1}$, the plant species accumulating the greatest amounts of arsenic. The following soil containing more arsenic was that corresponding to *Crupina vulgaris* (9218 $mg \cdot kg^{-1}$), also one of the most polluted plants. It seems to indicate that the grade of arsenic accumulation in plants depends directly on the extent of pollution of the site they grow. It would mean that the soil corresponding to *Dryopteris filix-mas* should be also one of the most contaminated ones. However, this does not occur and this soil is the least polluted among all (1260 $mg \cdot kg^{-1}$). Another exception could be the plant species *Quercus robur*, from Los Rueldos, whose arsenic concentration (32 and 44 $mg \cdot kg^{-1}$ in aerial part and roots respectively) were very low compared to the noticeable amount of arsenic in its corresponding soil (4452 $mg \cdot kg^{-1}$). This suggests that the amount of arsenic accumulated by the plant depends not only on the grade of pollution of the site, but also on the considered plants species and its ability to incorporate it. This is further discussed in section 3.4.

Similar arsenic concentrations to those of the plant species *Quercus robur* were found by Száková et al. (2010) [51], and González-Fernández et al. (2010) [52] in roots and leaves of beet plants growing in contaminated soils.

Table 6.1.1. Arsenic speciation in parts of different plant species collected in La Soterraña and in Los Rueldos mining sites. Results are presented as mean values ± standard deviation for three replicates.

Sampled site	Plant species	Plant part	Total As	As(III)		DMA		MMA		As(V)		Sum of the species	Identified species
			mg·kg^{-1}	mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%
Soterraña	*Crupina vulgaris*	Aerial part	167±5	22±2	13.0±0.7	0.39±0.02	0.23±0.01	0.94±0.05	0.56±0.02	122±2	73±3	145±3	87±4
		Roots	791±5	60±6	7.6±0.3	n.d.		n.d.		675±43	85±6	735±43	93±4
	Typha latifolia	Aerial part	6.0±1.0	1.2±0.1	20±1	0.67±0.01	11.2±0.1	1.17±0.03	20±0.07	3.7±0.1	62±1	6.7±0.3	113±1
		Roots	190±2	93±3	49±2	n.d.		4.20±0.05	2.2±0.1	71±4	37±1	168±5	88±1
	Dryopteris filix-mas	Aerial part	177±7	98±2	55±1	n.d.		n.d.		44±1	25±1	142±2	80±2
		Roots	377±6	69±3	18.3±0.5	n.d.		n.d.		192±8	51±1	261±9	69±2
Rueldos	*Quercus robur*	Aerial part	32±2	1.5±0.2	4.8±0.2	n.d.		n.d.		20±1	62±2	21±1	67±1
		Roots	44±1	4.8±0.1	10.8±0.5	n.d.		n.d.		31±1	71±3	36±1	83±4
	Calluna vulgaris	Leaves	359±2	13.6±0.1	3.8±0.1	n.d.		n.d.		232±19	65±3	246±19	69±3
		Stalks	150±11	3.05±0.02	2.0±0.1	n.d.		n.d.		86±9	57±4	89±9	59±2
		Woody roots	280±12	12.2±0.1	4.4±0.2	n.d.		n.d.		204±20	73±4	216±20	77±3
		Radicle roots	1217±13	8.7±0.2	0.71±0.02	n.d.		n.d.		776±44	64±3	785±44	65±3
	Dryopteris affinis	Leaves	77±3	2.8±0.1	3.6±0.2	n.d.		n.d.		50±4	65±3	53±4	68±3
		Stalks	46±1	2.2±0.1	4.8±0.2	n.d.		n.d.		29±1	62±3	31±1	67±2
		Roots	1371±4	4.9±0.2	0.36±0.01	n.d.		n.d.		453±21	33±3	902±41	66±2

n.d.: non detectable value

Table 6.1.2. Arsenic concentrations in the aerial part of every plant species, total arsenic concentrations in their corresponding soils and water-extractable, phosphate-extractable and available arsenic contents. Results are expressed as the mean value ± standard deviation for three replicates, in mg·kg^{-1} and the % extracted with regard to total arsenic content in the soil, in brackets.Transfer factor (TF) and transfer factor based on available arsenic in the soils (TF_{avail}).

PLANTS			SOILS				TRANSFER SOIL-PLANT	
Sampling area	**Plant species**	**As in aerial part**	**Total As**	**H_2O-extractable As**	**Phosphate-extractable As**	**Available As (H_2O+phosphate extractable)**	**Transfer factor (TF)**	**Transfer factor (available) (TF_{avail})**
Soterraña	***Crupina vulgaris***	167 ± 5	9218 ± 502	1373 ± 4 (14.9±0.1)	2057 ± 3 (22.3±0.1)	3430 ± 5 (37.2±0.1)	0.018	0.049
	Equisetum telmateia	8.9 ± 0.4	5623 ± 61	651 ± 8 (11.6±0.1)	1338 ± 13 (23.8±0.2)	1989 ± 15 (35.4±0.1)	0.0016	0.004
	Typha latifolia	6.0 ± 1.0	3680 ± 21	691 ± 21 (18.7±0.6)	874 ± 4 (23.7±0.1)	1565 ± 21 (42.4±0.7)	0.0016	0.004
	Dryopteris filix-mas	177 ± 7	1260 ± 8	159 ± 2 (12.6±0.1)	475 ± 9 (37.7±0.7)	634 ± 4 (50.3±0.6)	0.14	0.28
Rueldos	***Quercus robur***	32 ± 2	4452 ± 40	116 ± 4 (2.61±0.09)	270 ± 4 (6.05±0.08)	386 ± 10 (8.66±0.01)	0.0071	0.082
	Calluna vulgaris	359 ± 2	25901 ± 437	124 ± 1 (0.480±0.004)	486 ± 10 (1.88±0.04)	610 ± 10 (2.36±0.03)	0.0014	0.59
	Dryopteris affinis	77 ± 3	15423 ± 36	24.4 ± 0.6 (0.158±0.004)	1178 ± 8 (7.64±0.05)	1202 ± 8 (7.80±0.06)	0.0050	0.064

Regarding the arsenic distribution in the different parts of the plants (Table 6.1.1) clearly roots were responsible for accumulating most arsenic in all the studied plants (44-1371 $mg \cdot kg^{-1}$), as other authors found [35]. Likewise, radicle roots accumulated much more arsenic than woody roots, as it is evidenced by the results of *Calluna vulgaris,* whose radicle roots presented 1217 $mg \cdot kg^{-1}$ of arsenic while its woody roots contained 280 $mg \cdot kg^{-1}$. The lowest arsenic concentrations were found in aerial part (Table 6.1.1). In the cases that stalks and leaves could be separated, stalks accumulated lower arsenic concentrations than leaves. This behavior of concentrating more arsenic in roots is normal for non-hyperaccumulator plants, since they can not translocate arsenic from roots to their aerial parts in a great extent [13]. Conversely, hyperaccumulator plants present the majority of arsenic in the aboveground biomass [38, 53, 54].

6.1.3.2 Optimization of the extraction method for arsenic in plants

The extraction method reported by Bohari et al. (2002) [18], was used as starting conditions to extract arsenic species in the studied plants. This method consists on a microwave assisted digestion using a 0.3 M H_3PO_4 solution as extractant at 40 W for 20 min or 50 W for 6 min. This reagent shows higher efficiencies for the extraction of arsenic species from plant material than other extractants typically used, as water or methanol-water mixtures [16, 17, 55], since arsenic in terrestrial plants is mainly present as inorganic compounds and methods using polar organic solvents, developed to extract mainly organic arsenic species from marine samples, do not work well [18, 55-57]. The ability of H_3PO_4 to break S-As bonds could explain the higher extraction efficiencies [58]. Regarding this procedure, authors reported quantitative extractions when the proposed procedure was applied to the plant certified material CTA-VTL-2 (Virginia Tobacco Leaves) with a total arsenic content of 949 $\mu g \cdot kg^{-1}$. However, very low extraction efficiency was obtained (8.3%) when the extraction method was applied to *Calluna vulgaris* leaves material from Los Rueldos mine site. It has been reported that efficiencies obtained with a particular extraction method can depend on the sample matrix (different plant species or plant parts) and the amount of arsenic present in the sample [53, 59]. In this case, taking into account that tested samples contained about 378 times more arsenic than CRM Virginia Tobacco Leaves, extraction conditions were clearly insufficient in extracting arsenic species from Asturian plants, as they presented extremely high total arsenic concentrations. In order to improve extraction efficiencies a study was carried out testing different digestion procedures and conditions. Results are plotted in Fig. 6.1.2. Microwave assisted

extraction yielded low arsenic recoveries (8-56%) even at higher temperatures. Alternatively, a heat-assisted digestion procedure in open vessels on a graphite heating block (DP) was tested at increasing temperatures. The highest recoveries were obtained for 90 ºC. At this temperature, increasing extraction time to 60 min, recoveries of 80% were achieved whereas longer extraction times resulted in lower recoveries. Therefore, the 80% value obtained with DP 90 ºC and 60 min was considered the best procedure for the extraction of arsenic from Asturian plants. In addition, extraction assessment was performed applying the selected extraction to CRM Virginia Tobacco Leaves CTA-VTL-2. Quantitative recovery (94.7%) was obtained, which is agreement with results presented by Bohari et al. (2002) [18]. Finally, in order to assess the stability of arsenic species, the control plant was spiked with a mixture of the four main arsenic species [As(III), As(V), MMA and DMA]. Quantitative recoveries were obtained for the four spiked arsenic species, (94±2, 98±3, 96±3 and 102±4 % for As(III), As(V), MMA and DMA respectively), indicating that no interconversion of arsenic species occurred.

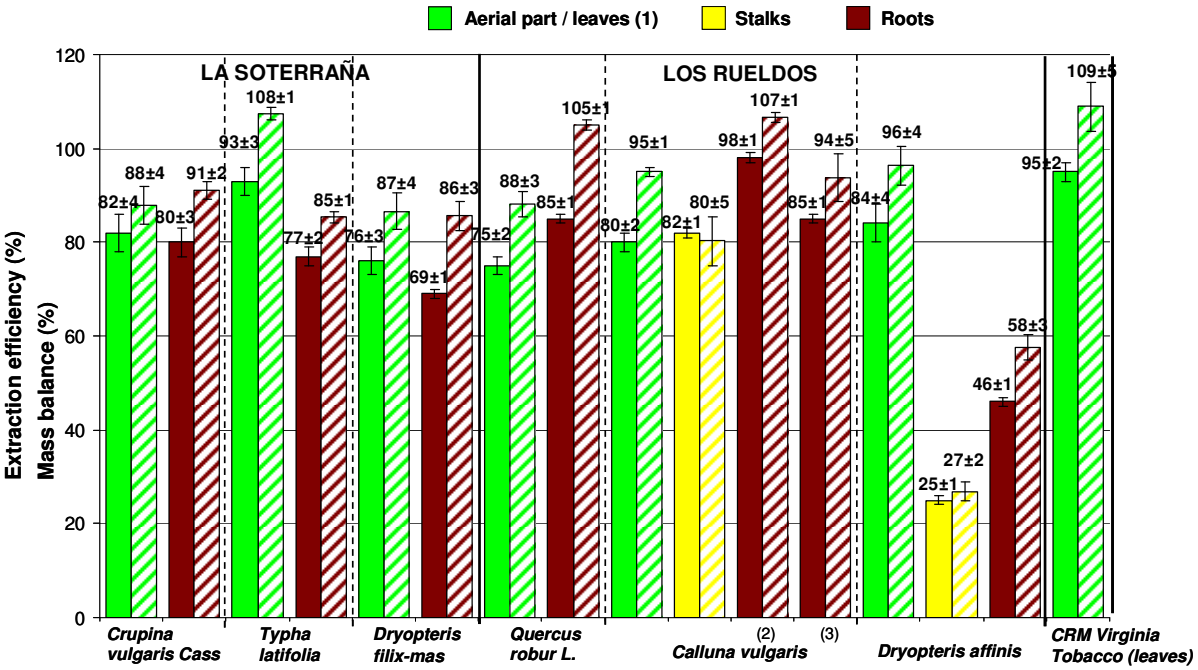

Fig. 6.1.3. Extraction efficiency and mass balance (in %). Bars in solid color correspond to extraction efficiency, whereas stripped bars correspond to mass balance. Standard deviation (for three replicates) is represented in every case.

(1) Aerial part is the term used for leaves + stalks when they were not separated. When they were separated, green color is for leaves and yellow for stalks.
(2) Woody roots.
(3) Radicle roots.

Once the extraction method was optimized, it was applied to every sample and extraction efficiencies were calculated in order to check the effectiveness of the method

in all the studied matrices. The extraction efficiencies (in %) were calculated as follows: [extracted As]·100/ [total As]. They ranged from 69±1 to 98±1% (Fig. 6.1.3) and were mostly above 80%.

6.1.3.3 Arsenic speciation in plants

The sum of identified species in the orthophosphoric acid extracts ranged from 65±3 to 113±1% of the total arsenic and were usually higher than 70% (Table 6.1.1), This means that both the extraction method and the chromatographic conditions used in this study work well with the studied samples.

Arsenate was the predominant species in almost all samples, representing usually more than 70% of the identified species. As(III) was present in all samples with percentages usually lower than 10%. There is no clear evidence of a greater abundance of As(III) in the aboveground biomass compared to other parts of the plant, as occurs in hyperaccumulator plants [36, 37, 60, 61]. DMA and MMA were scarcely found. They occurred in two plant species, in the aerial part of *Crupina vulgaris*, with contents lower than 1% for both species; and in *Typha latifolia*, whose contents in DMA and MMA accounted 11.2% and 20% respectively in aerial part and only 2.2% of MMA was present in roots

6.1.3.4 Arsenic uptake, translocation and bioaccumulation by the plant

Available arsenic in soils was considered as the sum of water-soluble and phosphate-extractable fractions. Since arsenate and arsenite pose high water solubility they can be leached by water at neutral pH and, as a consequence, this arsenic is immediately available [62]. In addition, phosphate is a good predictor of arsenic bioavailability since strong correlations have been reported between arsenic in plants and the extracted arsenic contents in soils with phosphate [27]. The better ability of phosphate to assess bioavailability can be attributed to its chemical similarity to arsenate. It has been established that phosphate competes with arsenate for soil adsorption sites [28-30].

Good correlations were found by other authors [27] between available arsenic and total arsenic in soils indicating that the amount of available arsenic depends largely on total arsenic. That is the case for soils from La Soterraña (R= 0.9958), but no correlation was found for soils from Los Rueldos (R= 0.0400).

Total arsenic contents in soils (Table 6.1.2) were very high in all cases, and were usually higher for Los Rueldos mining site soils. Despite this, the percentages of water-extractable arsenic, phosphate-extractable arsenic and the corresponding available arsenic fraction were much higher for soils from La Soterraña mining site. Lower availability of arsenic in Los Rueldos mining area could be explained by its higher iron contents [50] since it has been established that arsenates are strongly adsorbed onto Fe oxyhydroxides reducing its mobility, specially in acidic soils [21, 27, 63, 64].

Soil-to-plant transfer factor (TF) is currently used to evaluate the transfer ability of a metal from soil to plant in a given soil-plant system [65-68]. These values were calculated for arsenic in every case as follows: TF= As in the aerial part ($mg \cdot kg^{-1}$) / Total As in soil ($mg \cdot kg^{-1}$). When leaves and stalks were separated, the corresponding transfer factor was calculated taking into account only leaves. Studied plants showed TF values ranging from 0.0014 to 0.14 (table 2). Typical soil-to-plant TF for arsenic range from 0.01 to 0.1 [31]. No significant differences were found for TF values for both mining sites. The plant with the highest TF (0.14) was the fern *Dryopteris filix-mas* from La Soterraña. For hyperaccumulator plants, it is described that this factor should be greater than 1 [33], so in principle it seems that none of the studied plants have this property.

Another transfer factor, based on available arsenic content in soils (TF_{avail}) was also calculated. TF_{avail}= As in the aerial part ($mg \cdot kg^{-1}$) / Available As in soil ($mg \cdot kg^{-1}$). This factor measures the ability of a plant to accumulate a metal from the soil and transfer it to its aboveground biomass, reducing the contribution of soil-determined differences in mobility thereby emphasizing differences in the capacity of plant species to assimilate arsenic and translocate it to target organs [27]. Usually, a plant species is considered as hyperaccumulator when this factor is greater than 1 [34]. The two highest values (Table 6.1.2) were 0.28 and 0.59 for *Dryopteris filix-mas* and *Calluna vulgaris* respectively. The former plant species is a fern grown in La Soterraña. Despite the fact the soil where this plant grew is the least polluted, this species can accumulate high amounts of arsenic. Some authors found *Dryopteris filix-mas* suitable for phytoremediation in As-polluted sites [69] since TF_{avail} values were greater than 1. *Calluna vulgaris*, grown in Los Rueldos, has been described in literature as a hyperaccumulator plant and it is very common in contaminated sites [7, 70]. Although the TF value for this plant is very low (0.0014), the corrected TF_{avail} is much higher (0.59) but still far from those reported for hyperaccumulator plants [32, 34, 69].

According to Porter and Peterson [7], it can be attributed to the youth of the plant, since older leaves accumulate more arsenic than younger ones. Another explanation could be that the same species can accumulate very different amounts of arsenic depending on the site they grow and the source of the contamination [7]. It has been reported that arsenic coming from pesticides is usually more available than that coming from mining pollution [68]. In this case the available arsenic in soil is very low (2.36 %, Table 6.1.2). Furthermore, it has been established that transfer factors tend to decrease with increasing toxic metal concentrations in soils [27, 28]. Other authors would consider that this plant is not strictly a hyperaccumulator, but rather a tolerant one [32].

In plants from La Soterrraña certain relationship between arsenic concentration in the different parts of the plants and the concentration in their corresponding soils was observed. Significant correlations were found between arsenic in their aerial parts and the total arsenic contents in their soils (R= 0.9442) the water-extractable arsenic (R= 0.9978), the phosphate-extractable fraction (R= 0.9271), and the available arsenic in these soils (R= 0.9795) arsenic expected from TF results (Table 6.1.2) *Dryopteris filix-mas* did not fit this behavior as this plant species can translocate higher amounts of arsenic from soil to the aboveground biomass than the others. Regarding roots, plants from La Soterraña presented good correlations between arsenic contents in this part of the plant compared to total arsenic contents in their soils (R= 0.9995), the water-extractable contents (R= 0.9078), the phosphate extractable fraction (R= 0.9998) and the available arsenic in these soils (R= 0.9865). As before, the plant species *Dryopteris filix-mas* did not follow this trend as this plant tended to translocate the arsenic from roots to the aerial part. It has been reported that the reduced arsenic translocation and accumulation in non As-hyperaccumulating plants may be attributed to suppression of the high-affinity phosphate uptake system [41].

6.1.4. CONCLUSIONS

Our results suggested that arsenic extraction from plants containing extremely high total arsenic concentrations require stronger extraction conditions than those commonly applied for low-polluted plants. Extraction efficiencies above 80% were generally obtained with the proposed extraction method in all the plant samples investigated, regardless of the plant species, the parts of the plant or the total arsenic concentrations in the plant material. The selected chromatographic conditions allowed the qualitative and quantitative determination of all the arsenic species present in the extracts. In all the plants here investigated arsenic was present mostly as inorganic

As(V), although the more toxic species As(III) was also found, with specially significant contents in plants from La Soterraña. Low concentrations of DMA and MMA were only found in *Crupina vulgaris* and in *Typha latifolia*. There was not a meaningful difference of arsenic speciation among the different parts of the plants.

The studied plants accumulated extremely high amounts of arsenic in their tissues. Accumulation rates depended on both the grade of pollution of the site and on the plant species itself. Although arsenic concentrations were higher for soils from Los Rueldos, the bioavailability of this element was greater in La Soterraña mining site. The plant species with the highest TF_{avail} were *Dryopteris filix-mas*, from La Soterraña, and *Calluna vulgaris*, from Los Rueldos. That means better ability to accumulate and translocate arsenic from soils to their aerial parts. Both of them have been described as hyperaccumulator plants in literature, although in this study none of them reached transfer factors of 1 or greater.

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7. GENERAL CONCLUSIONS

Several conclusions arise from this research work; some of them concerning the analytical chemistry field, and some others as regards the environmental assessment performed in the area of study. On one hand, the analytical goals and innovations achieved through this work will be described, and on the other hand the conclusions from an environment point of view as regards the area of interest will be exposed.

CONCLUSIONS FROM AN ANALYTICAL POINT OF VIEW:

- A novel methodology for the study of arsenic fractionation in environmental solid samples has been developed and optimized. It is based on a seven-step sequential extraction procedure (SEP) and it intends to target the most relevant phases scavenging arsenic in soils and sediments, according to its mobility. Emphasis is placed on the most labile fractions, since arsenic retained on them may be easily mobilized and become bioavailable. Despite its novelty, it is a consolidated protocol whose extracting agents were selected following an extensive literature review and their selectivity was assured through experiments with natural and synthetic As-bearing minerals and As-spiked matrices. The usefulness of the proposed SEP for a wide variety of samples was proved by means of its application to real As-contaminated samples from different origins. Results showed that arsenic distributions were rather in accordance with the pollution source and the geochemical composition of each sample. In addition, the simplicity of the extraction steps of this protocol would easily allow the automation into a continuous flow scheme and it would be less time-consuming.

- From the comparison of the proposed SEP with those of BCR and Manful procedures it can be concluded the better suitability of the proposed scheme for the study of arsenic distribution in environmental solid samples, since it offers the most appropriate arsenic distribution pattern. This is due to: i) the consideration of the proposed protocol of the predominant anionic behaviour that this metalloid presents in nature ii) the careful selection of the main minerals phases retaining arsenic in environmental solid samples, and iii) the verified selectivity provided by all the extracting reagents.

- The results from the evaluation of different preservation ways for arsenic speciation in natural waters indicate that there is no significant difference in the sample conservation with the tested alternatives, since no alteration of the

original speciation occurred either for samples stored without any additive or for those preserved with HCl or EDTA acids along time.

- A new method for the extraction of the arsenic species As(V), As(III), MMA, and DMA from terrestrial plant material has been developed and optimized. The extraction was performed with 0.3 M orthophosphoric acid as extracting agent in a graphite block digestion system at 90 ºC for 1 hour, which can be considered very convenient due to its rapidity, cleanness and simplicity of the process. Quantitative extraction efficiency is achieved and it results appropriate for the diversity of plant tissues. The novelty of this method relies in the fact that it is suitable for samples containing extreme arsenic concentrations, since methods from the literature could not achieve quantitative recoveries for highly polluted samples. The extraction procedure was proved not to alter the original speciation as well as it was assured that no contamination or losses occurred during the extraction process. The selected chromatographic conditions allowed the identification and quantification of all the arsenic species present in the studied samples.

CONCLUSIONS FROM AN ENVIRONMENTAL POINT OF VIEW:

Soils and sediments

- Arsenic is widespread in La Soterraña, Los Rueldos and Santa Águeda mining areas, as the extreme arsenic concentrations found in soils and sediments of those sites confirm. However, where the encapsulation of the spoil heaps is performed, a notably decrease of arsenic contents is observed, as occurs in La Peña-El Terronal mine site.

- Arsenic partitioning obtained from the application of the proposed SEP to samples from the four studied mining areas is consistent with the sources of arsenic pollution, the mineralogy and geochemistry of the area and the extent of mining operations carried out in those sites. Iron oxyhydroxides, predominantly the amorphous forms, are the main phases retaining arsenic in practically all the studied areas, and especially in samples from Los Rueldos, affected by acid mine drainage. Samples from La Soterraña exhibit the highest arsenic mobility as a consequence of the intense ore treatment developed during decades in this mine site, posing an evident risk to the surroundings. Conversely, samples

from Santa Águeda mine present a higher proportion of arsenic associated with structural minerals, due to the lesser degree of exploitation of this mine. Nevertheless, it represents a long-term source of arsenic to the environment.

Waters

- Similarly to what occurs with soils and sediments, arsenic concentrations were very high in surrounding waters of La Soterraña and Los Rueldos mining sites, but significantly lower in waters from La Peña-El Terronal, due to the reduction of arsenic mobilization obtained with the encapsulation of the main spoil heap. Despite the lower arsenic contents in waters from La Peña-El Terronal, the high water flow involves an extremely high input of arsenic to the Caudal River, higher than the contribution of the other mines.

- The mobilization of arsenic from soils and sediments to waters is largely consistent with the mineralogy and the physicochemical characteristics of each area. Thus, arsenic release from calcareous areas to water, like those from La Soterraña mine site, is mainly explained by the solubilisation of Ca, Mg and Sr arsenates at alkaline pH close to neutrality. However, the solubilisation of As in waters from pyritic sites, as those from Los Rueldos mine, affected by acid mine drainage, is mostly attributed to the oxidation of As-bearing sulphur minerals and the dissolution of Al, Mn and Fe minerals retaining As. The highest arsenic concentrations were found in waters from La Soterraña mine. The acid pH of Los Rueldos provokes the solubilisation of heavy metals together with arsenic. Fortunately, arsenic and heavy metals concentrations decrease with distance to the focus and with pH increase, and at a medium distance the quality of waters notably improves.

- The presence of arsenic in the studied waters only occurs as inorganic species, and almost exclusively as As(V). The arsenic concentrations and speciation are no dependent on seasonal variations or the rainfall regime. The main physicochemical parameters governing arsenic mobility (pH, Eh) also remain constant along the year.

Plants

- The studied terrestrial plants from La Soterraña and Los Rueldos mining sites accumulate high arsenic contents in their tissues, being roots responsible for accumulating the bulk of arsenic. Arsenic occurs predominantly as inorganic species in the studied plants, regardless the part of the plant or the plant species. As(V) is the predominant species, although the presence of As(III) is also important, and only little amounts of DMA and MMA were found in some samples. The extent of arsenic accumulation by plants depends on both the bioavailable arsenic concentration in the soil and on the plant species in question.

- The sum of water and phosphate extractable arsenic fractions showed to be a reliable estimation of the bioavailable soil arsenic fraction for plants. From the determinations of total and bioavailable arsenic contents in their corresponding soils is inferred that despite soils from La Soterraña are less polluted than those from Los Rueldos, the bioavailability of arsenic in soils from La Soterraña is significantly higher.

- The determination of soil-to-plant transfer factors helps to discriminate the plant species with higher ability to accumulate and translocate arsenic from roots to its aerial part. In this study *Dryopteris filix-mas*, from La Soterraña, and *Calluna vulgaris*, from Los Rueldos, were the plants species with higher transfer factors and both of them are described in the literature as hyperaccumulator plants.

As a general conclusion it could be said that the analytical methodologies developed in this thesis allow the understanding of the forms of occurrence of arsenic in different compartments of the environment, such as soils and sediments, waters and terrestrial plants, and provide valuable tools in the assessment of the environmental fate of the areas of study.

Arsenic environmental impact is evident in all the studied areas. High arsenic concentrations are present in all the studied compartments of those ecosystems. The occurrence and mobility of arsenic is largely consistent with the mineralogical, geological and physicochemical characteristics of each mine site. Since prevention measures performed in certain mine sites resulted in a drastically decrease of As concentrations, isolation or sealing the spoil heaps of the other mines would be highly recommended, as well as the application of remediation techniques in order to reduce the extent of arsenic contamination.

Del presente trabajo de investigación se derivan varias conclusiones, algunas de ellas conciernen al campo de la química analítica y otras relativas a la evaluación medioambiental llevada a cabo en área de estudio. Así pues, primeramente se describirán los logros e innovaciones sobre metodologías analíticas y por otro lado, se expondrán las conclusiones medioambientales con respecto al área de interés.

CONCLUSIONES DESDE UN PUNTO DE VISTA ANALÍTICO:

- Se ha desarrollado y optimizado una nueva metodología para el estudio del fraccionamiento de arsénico en muestras sólidas medioambientales. Ésta se basa en un procedimiento de extracción secuencial (SEP) que comprende siete etapas y que tiene como objetivo la extracción sucesiva de las fases sólidas más importantes responsables de la retención de arsénico en suelos y sedimentos en función de su movilidad. Se hace especial énfasis en las fracciones más lábiles, ya que el arsénico retenido en ellas puede ser fácilmente movilizado y llegar a ser biodisponible. A pesar de tratarse de una metodología recientemente desarrollada, éste es un protocolo de extracción bien consolidado, ya que los agentes extractantes empleados han sido seleccionados tras una minuciosa revisión bibliográfica y la selectividad de éstos se ha asegurado mediante experimentos con matrices, tanto naturales como sintéticas, de minerales de arsénico y otras dopadas con arsénico. La viabilidad del método propuesto para una amplia variedad de muestras se ha contrastado mediante su aplicación a muestras reales contaminadas por arsénico procedentes de diversas fuentes de contaminación. Los resultados del fraccionamiento mostraron que las distribuciones de arsénico eran concordantes con el origen de la contaminación y con la composición geoquímica de cada muestra. Por otro lado, la simplicidad de las etapas de extracción de este protocolo permitirían fácilmente su automatización en un sistema de extracción de flujo continuo que requeriría mucho menos tiempo.

- De la comparación del método de extracción secuencial propuesto con los protocolos BCR y el desarrollado por Manful se puede concluir que el método propuesto resulta más idóneo para el estudio de la distribución de arsénico en muestras sólidas medioambientales, ya que ofrece un patrón de distribución del mismo más adecuado. Esto se debe a varias razones: i) la consideración por parte del método propuesto del comportamiento aniónico que presenta este metaloide en el medio ambiente, ii) la minuciosa selección de las principales

fases minerales responsables de la retención de arsénico, iii) la alta selectividad de todos los agentes extractantes, que ha sido verificada mediante diversas experiencias.

- Los resultados de la evaluación de las distintas formas de conservación de la especiación de arsénico en aguas indican que no hay diferencias significativas en la conservación de muestras con las alternativas ensayadas, ya que no hubo alteración con el tiempo de la especiación original ni en las muestras almacenadas sin aditivos ni en aquellas preservadas con HCl ó EDTA.

- Se ha desarrollado y optimizado un nuevo método para la extracción de las especies de arsénico As(V), As(III), MMA y DMA de tejidos vegetales de plantas terrestres. Ésta se lleva a cabo con ácido ortofosfórico 0.3 M como agente extractante, y se desarrolla en un sistema digestor de grafito a 90 ºC durante 1 hora, proceso que resulta muy conveniente debido a su rapidez, limpieza y sencillez. Con este procedimiento se logra eficazmente una extracción cuantitativa, y resulta adecuado para todos los tejidos vegetales. La novedad de este método se basa en el hecho de que resulta apropiado para muestras con concentraciones de arsénico muy elevadas, ya que los métodos propuestos en la bibliografía no logran una extracción cuantitativa para muestras muy contaminadas. Se comprobó que la extracción llevada a cabo con este procedimiento no altera la especiación original, al igual que se demostró que no ocurren contaminaciones o pérdidas durante el proceso. Por otro lado, las condiciones cromatográficas seleccionadas permitieron la identificación y cuantificación de todas las especies de arsénico presentes en las muestras estudiadas.

CONCLUSIONES DESDE UN PUNTO DE VISTA MEDIAMBIENTAL:

Suelos y sedimentos

- El arsénico se encuentra muy extendido en las zonas mineras de La Soterraña, Los Rueldos y Santa Águeda, como revelan las extremas concentraciones de arsénico encontradas en suelos y sedimentos de las áreas de estudio. En cambio, se observa una clara disminución de los contenidos de arsénico en los lugares en que se ha llevado a cabo un encapsulamiento de las escombreras, como sucede en el área minera de La Peña-El Terronal.

- El fraccionamiento de arsénico obtenido tras la aplicación del método de extracción secuencial propuesto a muestras procedentes de las cuatro áreas mineras de estudio es congruente con las fuentes de contaminación de arsénico, la mineralogía y geoquímica de cada área y el alcance de las labores mineras llevadas a cabo en estos emplazamientos. Los oxihidróxidos de hierro, y principalmente las formas amorfas, son las fases mineralógicas que retienen arsénico en mayor medida en prácticamente todas las áreas de estudio, especialmente en las muestras de Los Rueldos, afectadas por drenaje ácido de minas. Las muestras de la Soterraña presentan la mayor movilidad de arsénico, como consecuencia de la intensa labor de procesado del mineral llevado a cado durante décadas en este emplazamiento, lo que supone un evidente riesgo para su entorno. Por el contrario, las muestras de la mina Santa Águeda presentan una mayor proporción de arsénico asociado a minerales estructurales debido a que esta mina ha sido explotada en menor medida. No obstante, puede representar una fuente de arsénico para el medio ambiente a largo plazo.

Aguas

- Al igual que ocurre en suelos y sedimentos, las concentraciones en aguas circundantes a los yacimientos de La Soterraña y Los Rueldos fueron muy elevadas; en cambio, en aguas de La Peña-El Terronal éstas fueron significativamente menores, como consecuencia de la reducción de la movilización de arsénico obtenida tras el encapsulamiento de la principal escombrera. A pesar de estos contenidos de arsénico menores en aguas de La Peña-El Terronal, el elevado caudal de estas aguas conlleva un importante aporte de arsénico al río Caudal, superior a la contribución de las otras minas.

- La movilización de arsénico desde los suelos y sedimentos a las aguas es ampliamente concordante con la mineralogía y las características fisicoquímicas de cada área. De esta forma, la liberación de arsénico de zonas calcáreas a las aguas, como las de la mina La Soterraña, se explica fundamentalmente por la solubilización de arseniatos de Ca, Mg y Sr a pH alcalino cercano a la neutralidad. Sin embargo, la movilización de As en aguas de emplazamientos piríticos, como los de la mina de Los Rueldos, afectados por drenaje ácido de minas, se puede atribuir principalmente a la oxidación de minerales de sulfuros que contienen arsénico y la disolución de minerales de

Al, Mn y Fe que retienen arsénico. Las mayores concentraciones de arsénico se hallaron en aguas circundantes a la mina de La Soterraña. Por otro lado, el pH ácido de Los Rueldos provoca la disolución de metales pesados conjuntamente con el arsénico. Afortunadamente, las concentraciones de arsénico y metales pesados disminuyen con la distancia a la fuente de contaminación, el pH aumenta y la calidad de las aguas mejora notablemente a media distancia.

- La presencia de arsénico en las aguas estudiadas sólo se produce como especies inorgánicas, y casi exclusivamente como As(V). Ni las concentraciones de arsénico ni su especiación resultaron ser dependientes de variaciones estacionales ni del régimen de precipitaciones. Los principales parámetros fisicoquímicos que rigen la movilidad del arsénico (pH, Eh) también se mantienen constantes a lo largo del año.

Plantas

- Las plantas terrestres estudiadas de las zonas mineras de La Soterraña y Los Rueldos acumulan elevadas concentraciones de arsénico en sus tejidos, siendo las raíces responsables de la mayor parte de la acumulación. El arsénico se encuentra principalmente en forma de especies inorgánicas en las plantas estudiadas, con independencia de la parte de la planta de que se trate o de la especie de planta. El As(V) es la especie predominante, aunque la presencia de As(III) también es importante, y tan sólo se encontraron pequeñas cantidades de DMA y MMA en algunas muestras. El grado de acumulación de arsénico por parte de la planta depende tanto de la concentración de arsénico biodisponible en el suelo como de la especie de planta en cuestión.

- La suma de las fracciones de arsénico extraíbles con agua y fosfato de los suelos resultó ser una estimación fiable de la fracción de arsénico biodisponible para las plantas. De las determinaciones de los contenidos de arsénico total y biodisponible en los correspondientes suelos se infiere que a pesar de que los suelos de La Soterraña presentan menor contaminación que los de Los Rueldos, la biodisponibilidad de arsénico en suelos de La Soterraña es significativamente mayor.
- Los factores de transferencia suelo-planta aportan valiosa información para distinguir las especies de plantas con mayor capacidad para acumular y

translocar arsénico desde la raíz hasta la parte aérea. En este estudio, las especies de plantas *Dryopteris filix-mas*, de La Soterraña, and *Calluna vulgaris*, de la zona de Los Rueldos son las que presentan factores de transferencia más altos, y ambas han sido descritas en bibliografía como hiperacumuladoras.

Como conclusión general podría decirse que las metodologías analíticas desarrolladas en esta tesis permiten la comprensión de las formas de aparición de arsénico en los diferentes compartimentos estudiados del medio ambiente, tales como suelos y sedimentos, aguas y plantas terrestres, y proporcionan herramientas útiles para la evaluación del destino medioambiental de las áreas de estudio.

El impacto medioambiental del arsénico es evidente en todas las áreas de estudio. En todos los compartimentos estudiados de estos ecosistemas se encuentran concentraciones de arsénico muy elevadas. La presencia y movilidad del arsénico es ampliamente concordante con las características mineralógicas, geológicas y fisicoquímicas de cada emplazamiento minero. Dado que las medidas preventivas llevadas a cabo en algunas áreas mineras dieron como resultado una drástica disminución de las concentraciones de arsénico, se recomienda el aislamiento o sellado de las escombreras de las minas restantes, así como la aplicación de medidas de remediación que reduzcan el nivel de contaminación de arsénico.

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